(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 March 2002 (14.03.2002)

PCT

(10) International Publication Number
WO 02/20150 A2

(51) International Patent Classification[7]: B01J 19/00

(21) International Application Number: PCT/IB01/01650

(22) International Filing Date: 11 September 2001 (11.09.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/659,599 11 September 2000 (11.09.2000) US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US 09/659,599 (CIP)
Filed on 11 September 2000 (11.09.2000)

(71) Applicant (*for all designated States except US*): **AFFYMETRIX, INC.** [US/US]; 3380 Central Expressway, Santa Clara, CA 95051 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): **MCGALL, Glenn, H.** [US/US]; 1121 Sladky Avenue, Mountain View, CA 94040 (US). **BARONE, Anthony, D.** [US/US]; 2118 Ellen Avenue, San Jose, CA 95125 (US).

(74) Agents: **TREANNIE, Lisa, M.** et al.; Hamilton, Brook, Smith & Reynolds, P.C., 530 Virginia Road, P.O. Box 9133, Concord MA 01742-9133 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/20150 A2

(54) Title: PHOTOCLEAVABLE PROTECTING GROUPS

(57) Abstract: Novel compounds are provided which are useful as linking groups in chemical synthesis, preferably in the solid phase synthesis of oligonucleotides and polypeptides. The compounds are generally photolabile and comprise protecting groups which can be removed by photolysis to unmask a reactive group. The protecting groups has the general formula (Y), wherein: (Y) is a chemical structure as shown the Figure. Also provided is a method of forming, from component molecules, a plurality of compounds on a support, each compound occupying a separate predefined region of the support, using the protected compounds described above.

PHOTOCLEAVABLE PROTECTING GROUPS

RELATED APPLICATIONS

This application is a continuation-in-part of Application No. 09/659,599, filed September 11, 2000. The entire teachings of the above application are incorporated herein by reference.

BACKGROUND OF THE INVENTION

The present invention relates to the area of chemical synthesis. More particularly, this invention relates to photolabile compounds, reagents for preparing the same and methods for their use as photocleavable linkers and protecting groups, particularly in the synthesis of high density molecular arrays on solid supports. The use of a photolabile molecule as a linker to couple molecules to solid supports and to facilitate the subsequent cleavage reaction has received considerable attention during the last two decades. Photolysis offers a mild method of cleavage which complements traditional acidic or basic cleavage techniques. *See, e.g.*, Lloyd-Williams et al. (1993) *Tetrahedron* **49:**11065-11133. The rapidly growing field of combinatorial organic synthesis (*see, e.g.*, Gallop et al. (1994) *J. Med. Chem.* **37:**1233-1251; and Gordon et al. (1994) *J. Med. Chem.* **37:**1385-1401) involving libraries of peptides and small molecules has markedly renewed interest in the use of photolabile linkers for the release of both ligands and tagging molecules.

A variety of *ortho*-benzyl compounds as photolabile protecting groups have been used in the course of optimizing the photolithographic synthesis of both peptides (*see* Fodor et al. (1994) *Science* **251:**767-773) and oligonucleotides (*see* Pease et al. *Proc. Natl. Acad. Sci. USA* **91:**5022-5026). *See* PCT patent publication

Nos. WO 90/15070, WO 92/10092, and WO 94/10128; see also U.S. patent application Serial No. 07/971,181, filed 2 Nov. 1992, and Serial No. 08/310,510, filed September 22, 1994; Holmes et al. (1994) in *Peptides: Chemistry, Structure and Biology (Proceedings of the 13th American Peptide Symposium)*; Hodges et al. Eds.; ESCOM: Leiden; pp. 110-12, each of these references is incorporated herein by reference for all purposes. Examples of these compounds included the 6-nitroveratryl derived protecting groups, which incorporate two additional alkoxy groups into the benzene ring. Introduction of an α-methyl onto the benzylic carbon facilitated the photolytic cleavage with > 350 nm UV light and resulted in the formation of a nitroso-ketone.

Photocleavable protecting groups and linkers should be stable to a variety of reagents (*e.g.*, piperidine, TFA, and the like); be rapidly cleaved under mild conditions; and not generate highly reactive byproducts. The present invention provides such protecting groups and methods for their use in synthesizing high density molecular arrays.

SUMMARY OF THE INVENTION

According to a first aspect of the invention, novel compounds are provided which are useful for providing protecting groups in chemical synthesis, preferably in the solid phase synthesis of oligonucleotides and polypeptides. These compounds are generally photolabile and comprise protecting groups which can be removed by photolysis to unmask a reactive group. In one embodiment, the compounds have the general formulas as shown in Figure 1 and 9.

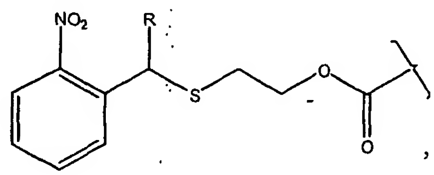
In another embodiment, compounds of the invention can be represented by structural formula I:

$$\text{Y-X}$$

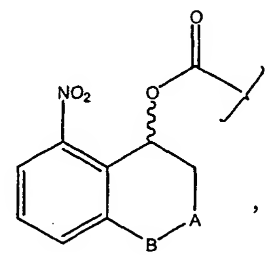
I.

In structural formula I, X is a leaving group or a compound having a masked reactive site, and Y is a photolabile protecting group. In one embodiment, the photolabile protecting group is bound to the masked reactive site. Therefore, the masked

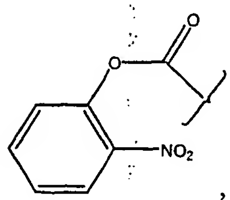
reactive site will not react with another compound until the photolabile protecting group is cleaved by, for example, exposure to radiation having a wavelength of greater than 350 nm. In a preferred embodiment, Y is selected from the group consisting of:



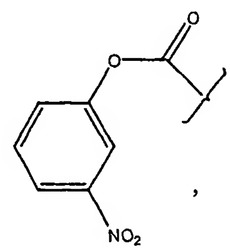
,



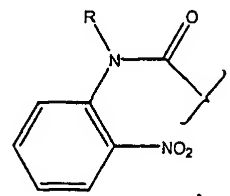
,



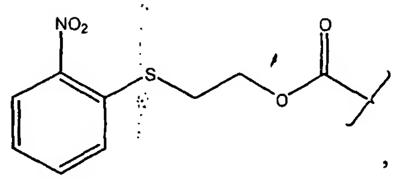
,



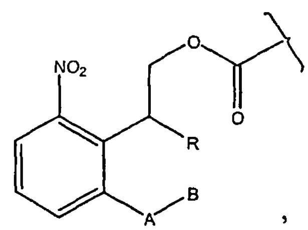
,



,

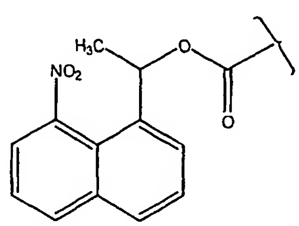


,



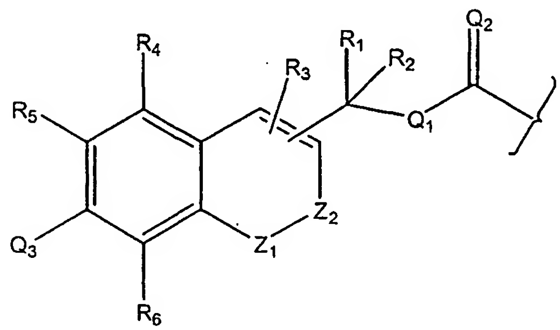
,

or



In the above group of structures, R is -H, an optionally substituted alkyl, or an optionally substituted aryl. A is -O-, -S-, -NR-, or $-(CH_2)_k-$. k is 0 or an integer from one to about three. B is a monovalent or divalent aprotic weakly basic group.

In another embodiment, compounds of the invention are represented by structural formula I, wherein Y is represented by structural formula II:

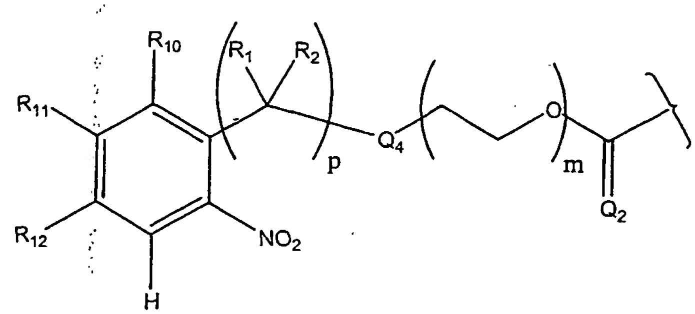


II.

In structural formula II, R_1 and R_2 are each, independently, -H, an optionally substituted alkyl, an optionally substituted alkenyl, an optionally substituted alkynyl, a trialkylsilyl, an optionally substituted aryl, an optionally substituted heteroaryl or a vinylogous derivative of the foregoing groups. Q_1 is –O-, -S-, $-CH_2O-$ or $-CH_2S-$. Q_2 is =O or =S. R_3 and R_4 are each, independently, -H, an optionally substituted alkyl, an optionally substituted aryl, an optionally substituted alkoxy, or $-NO_2$, provided that when one of R_3 or R_4 is $-NO_2$, at least one of R_1 or R_2 is –H. R_5 and R_6 are each, independently, -H, an optionally substituted alkyl, an optionally substituted aryl, or an optionally substituted alkoxy. Q_3 is –H, an optionally substituted alkoxy, or a dialkylamino. Z_1 and Z_2 taken together are –OC(O)-, -$NR_7C(O)$-, or $-CR_8=CR_9-$. R_7 is –H or an alkyl. R_8 is –H, an optionally substituted alkyl, an optionally substituted aryl, or an optionally substituted alkoxy. R_9 is –H, an optionally substituted alkyl, an optionally substituted aryl, or an optionally substituted alkoxy or $-NO_2$. Alternatively, R_8 and R_9, together with the carbon atoms to which they are attached, form a five or six membered carbocyclic or

heterocyclic ring. However, when none of R_3, R_4 or R_9 are $-NO_2$, Q_1 is not $-CH_2O-$ or $-CH_2S-$.

In another embodiment, compounds of the invention are represented by structural formula I, wherein Y is represented by structural formula III:



III.

In structural formula III, m is 0 or 1. p is 0, 1 or 2. R_1 and R_2 for each occurrence are, independently, -H, an optionally substituted alkyl, an optionally substituted alkenyl, an optionally substituted alkynyl, a trialkylsilyl, an optionally substituted aryl, an optionally substituted heteroaryl or a vinylogous derivative of the foregoing groups. Q_2 is =O or =S. Q_4 is –O-, -S-, or $-NR_{13}-$. R_{13} is –H, an optionally substituted alkyl or an optionally substituted aryl. R_{10} is –H, an optionally substituted alkyl, an optionally substituted aryl, an optionally substituted alkoxy or $-NO_2$. Alternatively, R_{10} and R_{13} together with the carbon atom and nitrogen atom to which they are form a five or six membered heterocycle. R_{11} and R_{12} are each, independently, -H, a halogen, an optionally substituted alkyl, an optionally substituted aryl, or an optionally substituted alkoxy. Alternatively, R_{11} and R_{12} taken together with the carbons to which they are attached form a five or six membered carbocycle or heterocycle.

Another aspect of this invention provides a method of attaching a molecule with a reactive site to a support comprising the steps of:

(a) providing a support with a reactive site;

(b) binding a molecule to the reactive site, the molecule comprising a masked reactive site attached to a photolabile protecting group of the formula as shown in Figure 1, and

(c) removing the photolabile protecting group to provide a derivatized support comprising the molecule with an unmasked reactive site immobilized thereon.

In another embodiment, the method of attaching a molecule with a reactive site to a support comprising the steps of:

(a) providing a support with a reactive site;

(b) reacting the reactive site of a first compound represented by structural formula I, wherein the compound represented by structural formula I further comprises a reactive site, with the support to form a bond; and

(c) removing the photolabile protecting group to provide a derivatized support comprising the compound of structural formula I with an unmasked reactive site immobilized thereon.

A related aspect of this invention provides a method of forming, from component molecules, a plurality of compounds on a support, each compound occupying a separate region of the support, said method comprising the steps of:

(a) activating a region of the support;

(b) binding a molecule to the region, said molecule comprising a masked reactive site linked to a photolabile protecting group of the formula as shown in Figure 1 or as in structural formula II or III;

(c) repeating steps (a) and (b) on other regions of the support whereby each of said other regions has bound thereto another molecule comprising a masked reactive site linked to the photolabile protecting group, wherein said another molecule may be the same or different from that used in step (b);

(d) removing the photolabile protecting group from one of the molecules bound to one of the regions of the support to provide a region bearing a molecule with an unmasked reactive site;

(e) binding an additional molecule to the molecule with an unmasked

reactive site;

(f) repeating steps (d) and (e) on regions of the support until a desired plurality of compounds is formed from the component molecules, each compound occupying separate regions of the support.

This method finds particular utility in synthesizing high density arrays of nucleic acids on solid supports in either the 3'->5' or 5'->3' directions.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a general outline of the alternative synthesis chemistries and outlines what the general structures for "Y" could be.

Figure 2 shows specific compounds that are preferred within the general structures shown in Fig. 1 and shows the stepwise yield when they were used to couple nucleotides together and the specific photolysis conditions used..

Figure 3 shows the synthesis of 5'-TEMPOC-T-Phosporamidite.

Figure 4 shows the synthesis of NINOC-T-CEP.

Figure 5 shows the synthesis of Me2NPOC-T-CEP. CEP stands for cyanoethyl N, N diisopropyl phosphoramidite.

Figure 6 shows the synthesis of Me3NPOC-T-CEP.

Figure 7 shows the synthesis of NP2NPOC-T-CEP.

Figure 8 shows the synthesis of NA1BOC-T-CEP.

Figure 9 shows the synthesis of 1-(3-nitrocoumarin-4-yl)ethyl alcohol.

Figure 10 shows the synthesis of 6,7-dimethoxycoumarin phosphoramidite. The method is also applicable to the synthesis of 7,8-dimethoxycoumarin phosphoramidite and 5,7-dimethoxycoumarin phosphoramidite

Figure 11 shows the synthesis of 7,8-dimethoxy-5-nitrocoumarinyl-4-ethanol.

Figure 12 shows the synthesis of (1,2)NNEOC-T-CEP.

Figure 13 shows the synthesis of (9,10)NPhenEOC-T-CEP.

Figure 14 shows the synthesis of 5'-(7-diethylaminocoumarin-3-yl)methyloxycarbonyl-T-CEP.

Figure 15 shows the synthesis of N-alkyl-4,5-substituted-2-nitroanalides.

Figure 16 shows the synthesis of (8,1)NNEOC-T-CEP.

Figure 17 shows the synthesis of 5'-(7-methoxy-3-nitrocoumarin-4-yloxycarbonyl)thymidine-3'-phosphoramidite.

Figure 18 shows the synthesis of (3,2)NNEOC-T-CEP.

Figure 19 shows the synthesis of 5'-(7-diethylaminocoumarin-4-yl)methyloxycarbonyl-T-CEP.

Figure 20 shows the synthesis of 5-bromo-7-nitroindolinylcarbonyl-T-CEP.

Figure 21 shows preferred “Y” groups.

DETAILED DESCRIPTION OF THE INVENTION

The following definitions are set forth to illustrate and define the meaning and scope of the various terms used to describe the invention herein.

The term "alkyl" refers to a branched or straight chain acyclic, monovalent saturated hydrocarbon radical of one to twenty carbon atoms.

The term “alkoxy” refers to an alkyl group that is attached to a compound via an oxygen.

The term "alkenyl" refers to an unsaturated hydrocarbon radical which contains at least one carbon-carbon double bond and includes straight chain, branched chain and cyclic radicals.

The term "alkynyl" refers to an unsaturated hydrocarbon radical which contains at least one carbon-carbon triple bond and includes straight chain, branched chain and cyclic radicals.

The term "aryl" refers to an aromatic monovalent carbocyclic radical having a single ring (e.g., phenyl) or two condensed rings (e.g., naphthyl), which can optionally be mono-, di-, or tri-substituted, independently, with alkyl, lower-alkyl, cycloalkyl, hydroxylower-alkyl, aminolower-alkyl, hydroxyl, thiol, amino, halo, nitro, lower-alkylthio, lower-alkoxy, mono-lower-alkylamino, di-lower-alkylamino, acyl, hydroxycarbonyl, lower-alkoxycarbonyl, hydroxysulfonyl, lower-alkoxysulfonyl, lower-alkylsulfonyl, lower-alkylsulfinyl, trifluoromethyl, cyano, tetrazoyl, carbamoyl, lower-alkylcarbamoyl, and di-lower-alkylcarbamoyl. Alternatively, two adjacent positions of the aromatic ring may be substituted with a

methylenedioxy or ethylenedioxy group. Typically, electron-donating substituents are preferred.

The term "heteroaromatic" or "heteroaryl" refers to an aromatic monovalent mono- or poly-cyclic radical having at least one heteroatom within the ring, *e.g.*, nitrogen, oxygen or sulfur, wherein the aromatic ring can optionally be mono-, di- or tri-substituted, independently, with alkyl, lower- alkyl, cycloalkyl, hydroxylower-alkyl, aminolower-alkyl, hydroxyl, thiol, amino, halo, nitro, lower-alkylthio, lower-alkoxy, mono-lower-alkylamino, di-lower-alkylamino, acyl, hydroxycarbonyl, lower-alkoxycarbonyl, hydroxysulfonyl, lower-alkoxysulfonyl, lower-alkylsulfonyl, lower-alkylsulfinyl, trifluoromethyl, cyano, tetrazoyl, carbamoyl, lower-alkylcarbamoyl, and di-lower-alkylcarbamoyl. For example, typical heteroaryl groups with one or more nitrogen atoms are tetrazoyl, pyridyl (*e.g.*, 4-pyridyl, 3-pyridyl, 2-pyridyl), pyrrolyl (*e.g.*, 2-pyrrolyl, 2-(N-alkyl)pyrrolyl), pyridazinyl, quinolyl (*e.g.* 2-quinolyl, 3-quinolyl etc.), imidazolyl, isoquinolyl, pyrazolyl, pyrazinyl, pyrimidinyl, pyridonyl or pyridazinonyl; typical oxygen heteroaryl radicals with an oxygen atom are 2-furyl, 3-furyl or benzofuranyl; typical sulfur heteroaryl radicals are thienyl, and benzothienyl; typical mixed heteroatom heteroaryl radicals are furazanyl and phenothiazinyl. Further the term also includes instances where a heteroatom within the ring has been oxidized, such as, for example, to form an N-oxide or sulfone.

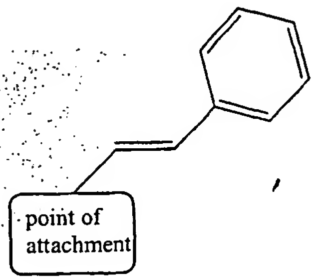
A heterocycloalkyl group, as used herein, is a non-aromatic ring system that preferably has five to six atoms and includes at least one heteroatom selected from nitrogen, oxygen, and sulfur. Examples of heterocyclalkyl groups include morpholinyl, piperidinyl, piperazinyl, thiomorpholinyl, pyrrolidinyl, thiazolidinyl, tetrahydrothienyl, azetidinyl, tetrahydrofuryl, dioxanyl and dioxepanyl.

The term "heterocycle" includes a heteroaryl groups and heterocycloalkyl groups.

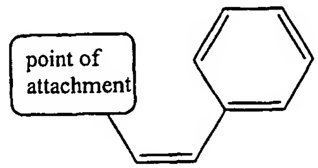
The term "carbocycle" includes cycloalkyl groups having from 3 to 10 carbon atoms and aryl groups.

The term "vinylogous derivative" refers to a group that is attached to a compound by a vinyl group. The vinyl group can have either a cis or trans

configuration. For example, a trans and a cis vinylogous derivative of a phenyl group would have the following structural formulas:



or



The term "optionally substituted" refers to the presence or lack thereof of a substituent on the group being defined. When substitution is present the group may be mono-, di- or tri-substituted, independently, with alkyl, lower-alkyl, cycloalkyl, hydroxylower-alkyl, aminolower-alkyl, hydroxyl, thiol, amino, halo, nitro, lower-alkylthio, lower-alkoxy, mono-lower-alkylamino, di-lower-alkylamino, acyl, hydroxycarbonyl, lower-alkoxycarbonyl, hydroxysulfonyl, lower-alkoxysulfonyl, lower-alkylsulfonyl, lower-alkylsulfinyl, trifluoromethyl, cyano, tetrazoyl, carbamoyl, lower-alkylcarbamoyl, and di-lower-alkylcarbamoyl. Typically, electron-donating substituents such as alkyl, lower-alkyl, cycloalkyl, hydroxylower-alkyl, aminolower-alkyl, hydroxyl, thiol, amino, halo, lower-alkylthio, lower-alkoxy, mono-lower-alkylamino and di-lower-alkylamino are preferred.

The term "electron donating group" refers to a radical group that has a lesser affinity for electrons than a hydrogen atom would if it occupied the same position in the molecule. For example, typical electron donating groups are hydroxy, alkoxy (e.g. methoxy), amino, alkylamino and dialkylamin0.

The term "leaving group" means a group capable of being displaced by a nucleophile in a chemical reaction, for example halo, nitrophenoxy, pentafluorophenoxy, alkyl sulfonates (*e.g.*, methanesulfonate), aryl sulfonates, phosphates, sulfonic acid, sulfonic acid salts, and the like.

"Activating group" refers to those groups which, when attached to a particular functional group or reactive site, render that site more reactive toward covalent bond formation with a second functional group or reactive site. The group of activating groups which are useful for a carboxylic acid include simple ester groups and anhydrides. The ester groups include alkyl, aryl and alkenyl esters and in particular such groups as 4-nitrophenyl, N-hydroxylsuccinimide and pentafluorophenol. Other activating groups are known to those of skill in the art.

"Chemical library" or "array" is an intentionally created collection of differing molecules which can be prepared either synthetically or biosynthetically and screened for biological activity in a variety of different formats (*e.g.*, libraries of soluble molecules; and libraries of compounds tethered to resin beads, silica chips, or other solid supports). The term is also intended to refer to an intentionally created collection of stereoisomers.

"Predefined region" refers to a localized area on a solid support which is, was, or is intended to be used for formation of a selected molecule and is otherwise referred to herein in the alternative as a "selected" region. The predefined region may have any convenient shape, *e.g.*, circular, rectangular, elliptical, wedge-shaped, etc. For the sake of brevity herein, "predefined regions" are sometimes referred to simply as "regions." In some embodiments, a predefined region and, therefore, the area upon which each distinct compound is synthesized smaller than about 1 cm^2 or less than 1 mm^2. Within these regions, the molecule synthesized therein is preferably synthesized in a substantially pure form. In additional embodiments, a predefined region can be achieved by physically separating the regions (*i.e.*, beads, resins, gels, etc.) into wells, trays, etc.

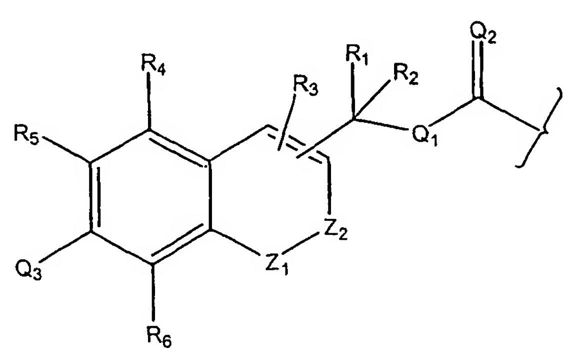
"Solid support", "support", and "substrate" refer to a material or group of materials having a rigid or semi-rigid surface or surfaces. In many embodiments, at least one surface of the solid support will be substantially flat, although in some embodiments it may be desirable to physically separate synthesis regions for different compounds with, for example, wells, raised regions, pins, etched trenches, or the like. According to other embodiments, the solid support(s) will take the form of beads, resins, gels, microspheres, or other geometric configurations.

Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification procedure such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography, thick-layer (preparative) chromatography, distillation, or a combination of these procedures. Specific illustrations of suitable separation and isolation procedures can be had by references to the examples hereinbelow. However, other equivalent separation or isolation procedures can, or course, also be used.

A "channel block" is a material having a plurality of grooves or recessed regions on a surface thereof. The grooves or recessed regions may take on a variety of geometric configurations, including but not limited to stripes, circles, serpentine paths, or the like. Channel blocks may be prepared in a variety of manners, including etching silicon blocks, molding or pressing polymers, etc.

This invention provides novel compounds which are useful for providing protecting groups in chemical synthesis, preferably in the solid phase synthesis of oligonucleotides and polypeptides and high density arrays thereof. These compounds are generally photolabile and comprise protecting groups which can be removed by photolysis to unmask a reactive group. Specifically, the preferred compounds are shown in Figures 1 and 9. More specifically, the preferred compounds have R or R1 groups which can be H, optionally substituted alkyl, alkenyl, alknyl, aryl, or heteroaromatic groups.

In another embodiment, compounds of the invention are represented by structural formula I, wherein Y is represented by structural formula II:

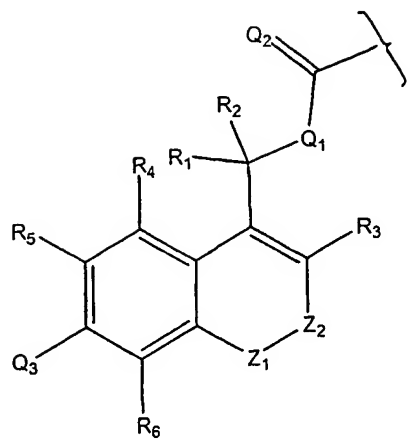


II.

In structural formula II, R_1 and R_2 are each, independently, -H, an optionally substituted alkyl, an optionally substituted alkenyl, an optionally substituted alkynyl, a trialkylsilyl, an optionally substituted aryl, an optionally substituted heteroaryl or a vinylogous derivative of the foregoing groups. Q_1 is –O-, -S-, $-CH_2O-$ or $-CH_2S-$. Q_2 is =O or =S. R_3 and R_4 are each, independently, -H, an optionally substituted alkyl, an optionally substituted aryl, an optionally substituted alkoxy, or $-NO_2$, provided that when one of R_3 or R_4 is $-NO_2$, at least one of R_1 or R_2 is –H. R_5 and R_6 are each, independently, -H, an optionally substituted alkyl, an optionally substituted aryl, or an optionally substituted alkoxy. Q_3 is –H, an optionally substituted alkoxy, or a dialkylamino. Z_1 and Z_2 taken together are –OC(O)-, -$NR_7C(O)$-, or $-CR_8=CR_9-$. R_7 is –H or an alkyl. R_8 is –H, an optionally substituted alkyl, an optionally substituted aryl, or an optionally substituted alkoxy. R_9 is –H, an optionally substituted alkyl, an optionally substituted aryl, or an optionally substituted alkoxy or $-NO_2$. Alternatively, R_8 and R_9, together with the carbon atoms to which they are attached, form a five or six membered carbocyclic or heterocyclic ring. However, when none of R_3, R_4 or R_9 are $-NO_2$, Q_1 is not $-CH_2O-$ or $-CH_2S-$.

In a preferred embodiment, X is a compound having a masked reactive site and further comprises a reactive site. More preferably, X is selected from the group consisting of an amino acid, a nucleoside, a nucleoside phosphoramidite, a nucleoside H-phosphonate, a nucleotide, a solid support, a peptide, an oligonucleotide, a protein, a hormone, an antibody, a polysaccharide, a monosaccharide, a disaccharide, a solid support bound peptide, a solid support bound oligonucleotide, a solid support bound protein, a solid support bound hormone, a solid support bound antibody, a solid support bound polysaccharide, a solid support bound monosaccharide, or a solid support bound disaccharide.

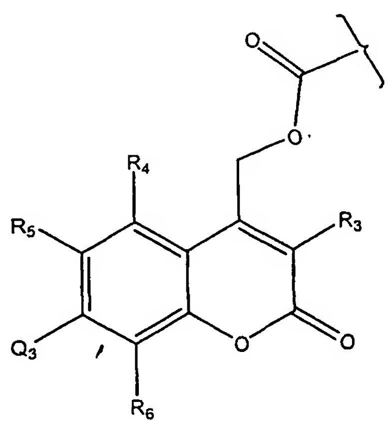
In another preferred embodiment, Y is represented by structural formula IV:



IV.

In structural formula IV, Q_1, Q_2, Q_3, R_1, R_2, R_3, R_4, R_5, R_6, Z_1 and Z_2 are defined as above.

More preferably, Y is represented by structural formula V:



V.

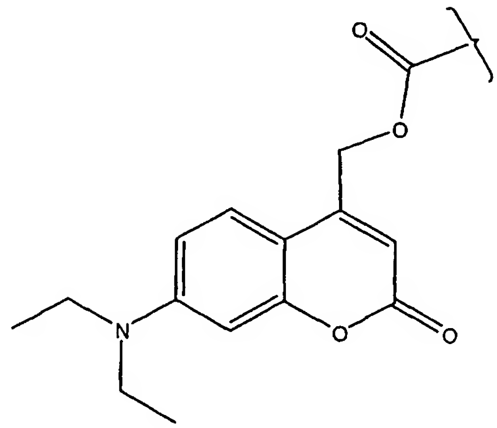
In structural formula V, Q_2, Q_3, R_3, R_4, R_5, and R_6 are defined as above.

In structural formulas II, IV, and V, one of R_3 or R_4 is, preferably, $-NO_2$.

Preferably, in structural formula V, R_3, R_4, R_5 and R_6 are $-H$ and Q_3 is a

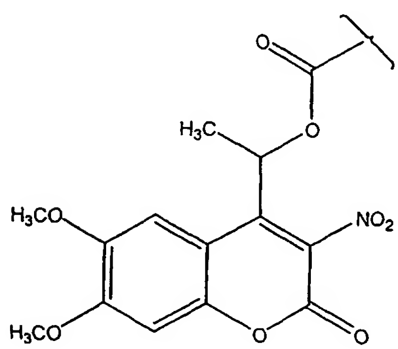
dialkylamino.

In another preferred embodiment, Y is represented by structural formula VI:

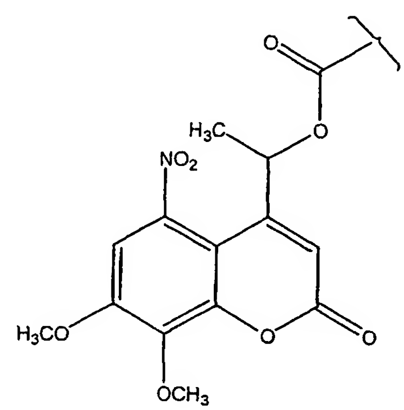


VI.

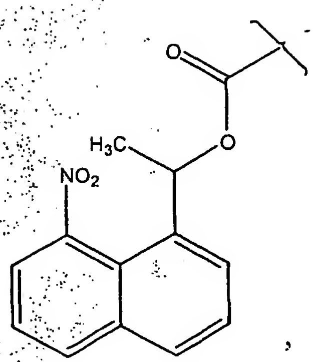
In another embodiment, Y is selected from the group consisting of:



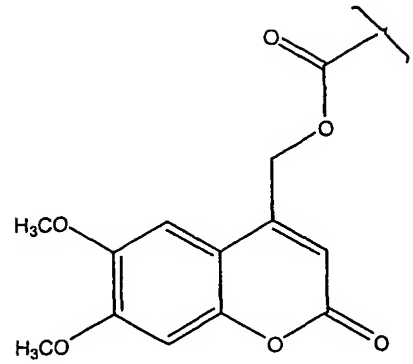
,



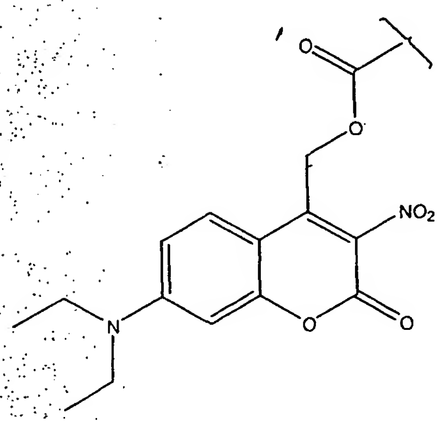
,



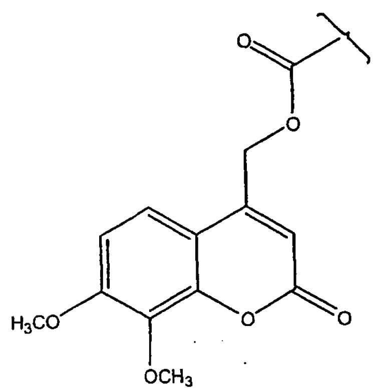
,



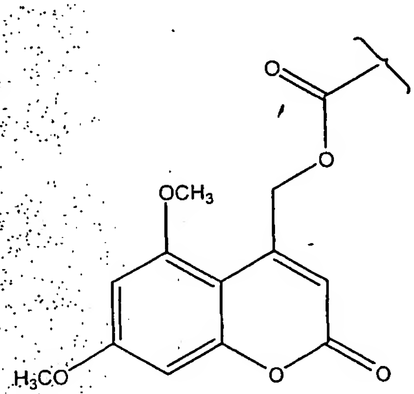
,



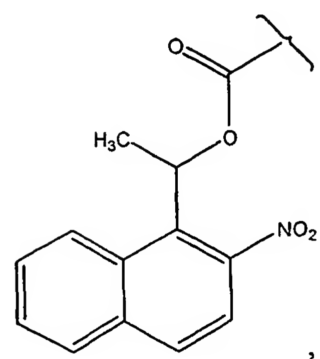
,



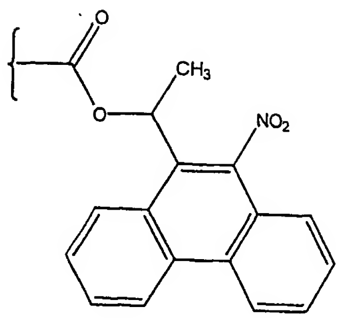
,



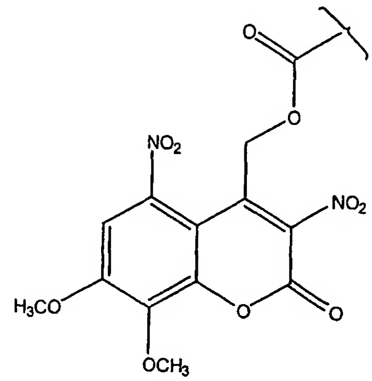
,



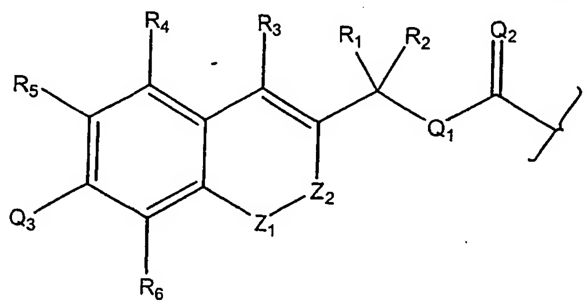
,



and



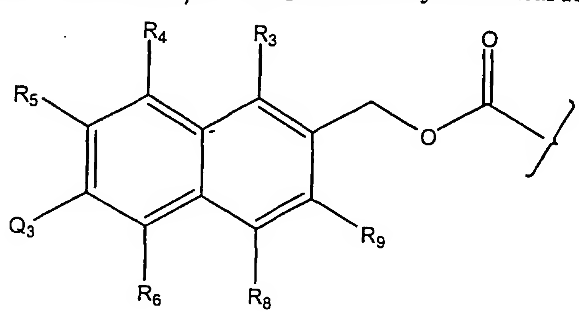
In another embodiment, Y is a group represented by structural formula VII:



VII.

In structural formula VII, Q_1, Q_2, Q_3, R_1, R_2, R_3, R_4, R_5, R_6, Z_1 and Z_2 are defined as above.

In another embodiment, Y is represented by structural formula VIII:

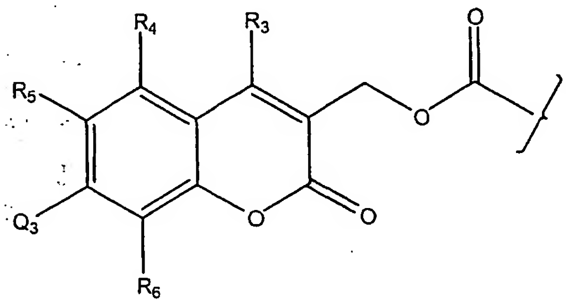


VIII.

In structural formula VIII, Q_3, R_3, R_4, R_5, R_6, R_8, and R_9 are defined as above.

Preferably, in structural formula VIII, R_3 or R_9 is $-NO_2$.

In another embodiment, Y is represented by structural formula IX:



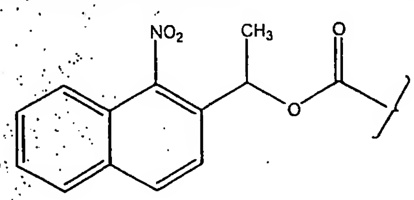
IX.

,

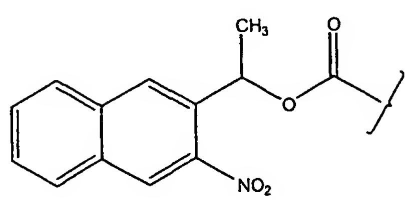
In structural formula IX, Q_3, R_3, R_4, R_5, and R_6 are defined as above.

In structural formula IX, R_3, R_4, R_5 and R_6 are preferably –H and Q_3 is preferably a dialkylamino.

In another embodiment, Y is selected from the group consisting of:

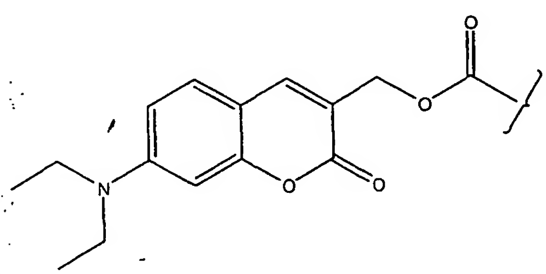


,

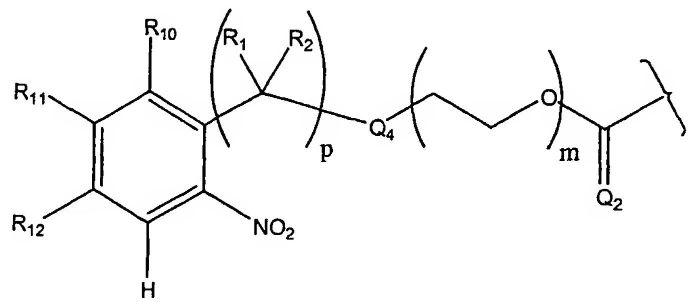


,

and



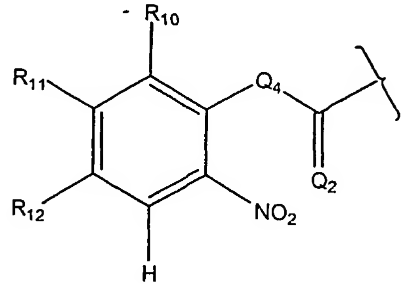
In another embodiment, compounds of the invention are represented by structural formula I, wherein Y is represented by structural formula III:



III.

In structural formula III, m is 0 or 1. p is 0, 1 or 2. R_1 and R_2 for each occurrence are, independently, -H, an optionally substituted alkyl, an optionally substituted alkenyl, an optionally substituted alkynyl, a trialkylsilyl, an optionally substituted aryl, or an optionally substituted heteroaryl. Q_2 is =O or =S. Q_4 is –O-, -S-, or -NR_{13}-. R_{13} is –H, an optionally substituted alkyl or an optionally substituted aryl. R_{10} is –H, an optionally substituted alkyl, an optionally substituted aryl, an optionally substituted alkoxy or –NO_2. Alternatively, R_{10} and R_{13} together with the carbon atom and nitrogen atom to which they are form a five or six membered heterocycle. R_{11} and R_{12} are each, independently, -H, a halogen, an optionally substituted alkyl, an optionally substituted aryl, or an optionally substituted alkoxy. Alternatively, R_{11} and R_{12} taken together with the carbons to which they are attached form a five or six membered carbocycle or heterocycle.

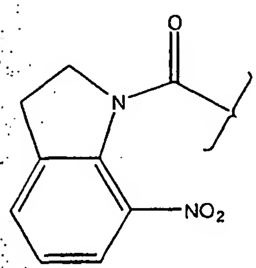
In one embodiment, m and p of structural formula III are both 0 and Y is represented by structural formula X:



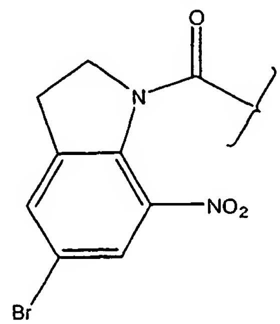
X.

In structural formula X, Q_2, Q_4, R_{10}, R_{11}, and R_{12} are defined as above.

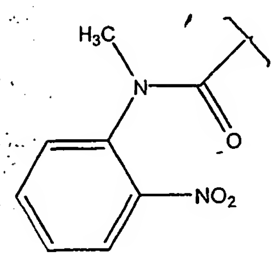
In a preferred embodiment, Y is selected from the group consisting of:



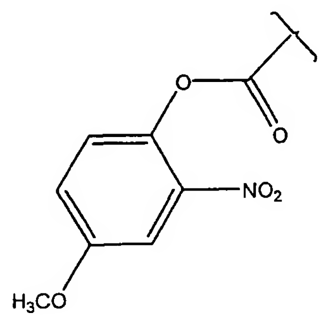
,



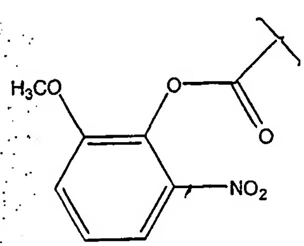
,



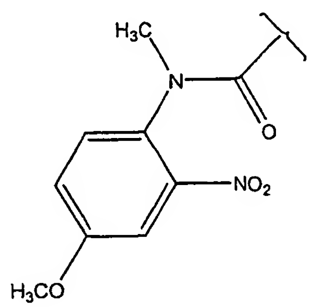
,



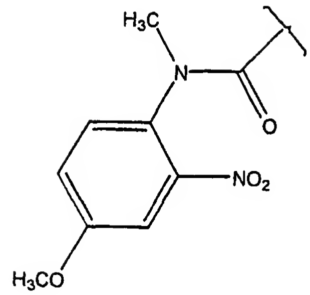
,



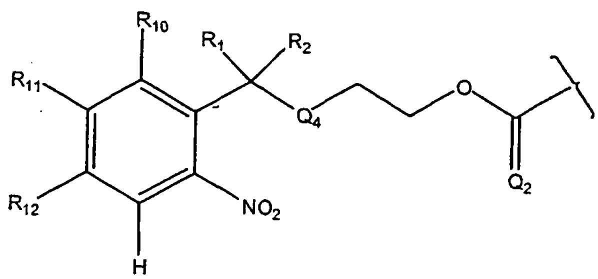
,



,

and 

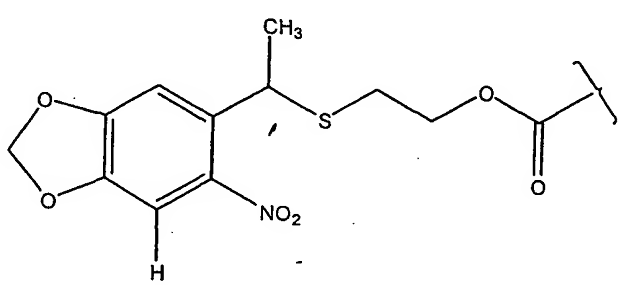
In another embodiment, in structural formula III, m is 1 and p is 1 and Y is represented by structural formula XI:



XI.

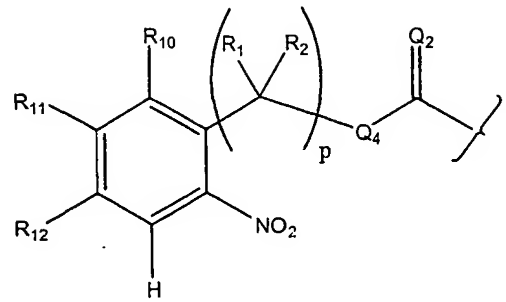
In structural formula XI, Q_2, Q_4, R_1, R_2, R_{10}, R_{11}, and R_{12} are defined as above.

In a preferred embodiment, Y is represented by structural formula XII:



XII.

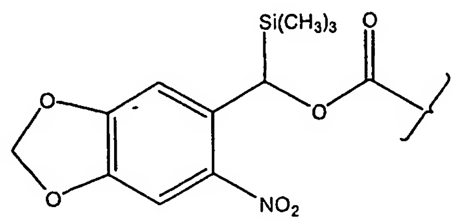
In another embodiment, in structural formula III, m is 0 and p is 1 or 2, and Y is represented by structural formula XIII:



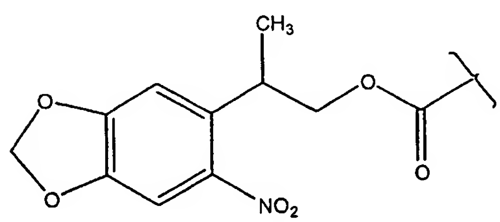
XIII.

In structural formula XIII, Q_2, Q_4, R_1, R_2, R_{10}, R_{11}, and R_{12} are defined as above.

In a preferred embodiment, Y is selected from the group consisting of:



and



Thus, the reagents comprising the protecting groups recited above can be used in numerous applications where protection of a reactive nucleophilic group is required. Such applications include, but are not limited to polypeptide synthesis, both solid phase and solution phase, oligo- and polysaccharide synthesis, polynucleotide

synthesis, protection of nucleophilic groups in organic syntheses of potential drugs, etc.

Preferably, M will be a monomeric building block that can be used to make a macromolecule. Such building blocks include amino acids, nucleic acids, nucleotides, nucleosides, monosaccharides and the like. Preferred nucleosides are deoxyadenosine, deoxycytidine, thymidine and deoxyguanosine as well as oligonucleotides incorporating such nucleosides. Preferably, the building block is linked to the photolabile protecting group via a hydroxy or amine group. When nucleotide and oligonucleotide compositions are used, with the protecting groups of this invention, the protecting groups are preferably incorporated into the 3'-OH or the 5'-OH of the nucleoside. Other preferred compounds are protected peptides, proteins, oligonucleotides and oligodeoxynucleotides. Small organic molecules, proteins, hormones, antibodies and other such species having nucleophilic reactive groups can be protected using the protecting groups disclosed herein.

The use of nucleoside and nucleotide analogs is also contemplated by this invention to provide oligonucleotide or oligonucleoside analogs bearing the protecting groups disclosed herein. Thus the terms nucleoside, nucleotide, deoxynucleoside and deoxynucleotide generally include analogs such as those described herein. These analogs are those molecules having some structural features in common with a naturally occurring nucleoside or nucleotide such that when incorporated into an oligonucleotide or oligonucleoside sequence, they allow hybridization with a naturally occurring oligonucleotide sequence in solution. Typically, these analogs are derived from naturally occurring nucleosides and nucleotides by replacing and/or modifying the base, the ribose or the phosphodiester moiety. The changes can be tailor made to stabilize or destabilize hybrid formation or enhance the specificity of hybridization with a complementary nucleic acid sequence as desired.

Analogs also include protected and/or modified monomers as are conventionally used in oligonucleotide synthesis. As one of skill in the art is well aware oligonucleotide synthesis uses a variety of base-protected deoxynucleoside derivatives in which one or more of the nitrogens of the purine and pyrimidine

moiety are protected by groups such as dimethoxytrityl, benzyl, tert-butyl, isobutyl and the like. Specific monomeric building blocks which are encompassed by this invention include base protected deoxynucleoside H-phosphonates and deoxynucleoside phosphoramidites.

For instance, structural groups are optionally added to the ribose or base of a nucleoside for incorporation into an oligonucleotide, such as a methyl, propyl or allyl group at the 2'-0 position on the ribose, or a fluoro group which substitutes for the 2'-O group, or a bromo group on the ribonucleoside base. 2'-O-methyloligoribonucleotides (2'-O-MeORNs) have a higher afinity for complementary nucleic acids (especially RNA) than their unmodified counterparts. 2'-0-MeORNA phosphoramidite monomers are available commercially, *e.g.*, from Chem Genes Corp. or Glen Research, Inc. Alternatively, deazapurines and deazapyrimidines in which one or more N atoms of the purine or pyrimidine heterocyclic ring are replaced by C atoms can also be used.

The phosphodiester linkage, or "sugar-phosphate backbone" of the oligonucleotide analogue can also be substituted or modified, for instance with methyl phosphonates or O-methyl phosphates. Another example of an oligonucleotide analogue for purposes of this disclosure includes "peptide nucleic acids" in which a polyamide backbone is attached to oligonucleotide bases, or modified oligonucleotide bases. Peptide nucleic acids which comprise a polyamide backbone and the bases found in naturally occurring nucleosides are commercially available.

Nucleotides with modified bases can also be used in this invention. Some examples of base modifications include 2-aminoadenine, 5-methylcytosine, 5-(propyn-1-yl)cytosine, 5-(propyn-1-yl)uracil, 5-bromouracil, and 5-bromocytosine which can be incorporated into oligonucleotides in order to increase binding affinity for complementary nucleic acids. Groups can also be linked to various positions on the nucleoside sugar ring or on the purine or pyrimidine rings which may stabilize the duplex by electrostatic interactions with the negatively charged phosphate backbone, or through hydrogen bonding interactions in the major and minor groves. For example, adenosine and guanosine nucleotides can be substituted at the N^2

position with an imidazolyl propyl group, increasing duplex stability. Universal base analogues such as 3-nitropyrrole and 5-nitroindole can also be included. A variety of modified oligonucleotides and oligonucleotide analogs suitable for use in this invention are described "Antisense Research and Applications", S.T. Crooke and B. LeBleu (eds.) (CRC Press, 1993) and "Carbohydrate Modifications in Antisense Research" in ACS Symp. Ser. #580, Y.S. Sanghvi and P.D. Cook (eds.) ACS, Washington, D.C. 1994).

Compounds of this invention can be prepared by carbonylating an alcohol or amine precursor of "Y" with a carbonylation reagent such as for example, phosgene ($COCl_2$), carbonyldiimidazole or pentafluorophenoxy chloroformate and the like to provide Y_1-C(O)-X wherein Y_1-C(O)- is a Y group, and X is a leaving group derived from the carbonylating reagent (Cl, if phosgene was used, pentafluorophenoxy, if pentafluorophenoxy chloroformate was used, etc.). This intermediate, Y_1-C(O)-X is then reacted with a molecule M carrying a nucleophilic group whose protection is desired to yield a protected building block Y_1-C(O)-M.

Alternatively, one may first carbonylate the group on the molecule being protected with a carbonylation reagent, such as one described above, and subsequently displace the leaving group X thus inserted with the hydroxyl group of the aromatic carbinol. In either procedure, one frequently uses a base such as triethylamine or diisopropylethylamine and the like to facilitate the displacement of the leaving group.

One of skill in the art will recognize that the protecting groups disclosed herein can also be attached to species not traditionally considered as "molecules". Therefore, compositions such as solid surfaces (*e.g.*, paper, nitrocellulose, glass, polystyrene, silicon, modified silicon, GaAs, silica and the like), gels (*e.g.*, agarose, sepharose, polyacrylamide and the like to which the protecting groups disclosed herein are attached are also contemplated by this invention.

The protecting groups of this invention are typically removed by photolysis, i.e. by irradiation, though in selected cases it may be advantageous to use acid or base catalyzed cleavage conditions. The synthesis can occur in either the 3'>5' or 5'>3' directions. Generally irradiation is at wavelengths greater than about 350 nm,

preferably at about 365 nm. The photolysis is usually conducted in the presence of hydroxylic solvents, such as aqueous, alcoholic or mixed aqueous-alcoholic or mixed aqueous-organic solvent mixtures. Alcoholic solvents frequently used include methanol and ethanol. The photolysis medium may also include nucleophilic scavengers such as hydrogen peroxide. Photolysis is frequently conducted at neutral or basic pH.

This invention also provides a method of attaching a molecule with a reactive site to a support, comprising the steps of:

(a) providing a support with a reactive site;

(b) binding a molecule to the reactive site, said first molecule comprising a masked reactive site attached to a photolabile protecting group of the formula Y, and

(c) removing the photolabile protecting group to provide a derivatized support comprising the molecule with an unmasked reactive site immobilized thereon.

As one of skill will recognize, the process can be repeated to generate a compound comprising a chain of component molecules attached to the solid support. In a "mix and match" approach, the photolabile protecting groups may be varied at different steps in the process depending on the ease of synthesis of the protected precursor molecule. Alternatively, photolabile protecting groups can be used in some steps of the synthesis and chemically labile (e.g. acid or base sensitive groups) can be used in other steps, depending for example on the availability of the component monomers, the sensitivity of the substrate and the like. This method can also be generalized to be used in preparing arrays of compounds, each compound being attached to a different and identifiable site on the support as is disclosed in U.S. Patent Nos. 5,143,854, 5,384,261, 5,424,186 5,445,934, 6,022963 and copending U.S. Patent Application, Serial No. 08/376,963, filed January 23, 1995, incorporated for reference for all purposes in their entireties.

As one of skill will recognize, the process can be repeated to generate a compound comprising a chain of component molecules attached to the solid support. In a "mix and match" approach, the photolabile protecting groups may be varied at different steps in the process depending on the ease of synthesis of the protected

precursor molecule. Alternatively, photolabile protecting groups can be used in some steps of the synthesis and chemically labile (e.g. acid or base sensitive groups) can be used in other steps, depending for example on the availability of the component monomers, the sensitivity of the substrate and the like. This method can also be generalized to be used in preparing arrays of compounds, each compound being attached to a different and identifiable site on the support as is disclosed in U.S. Pat. Nos. 5,143,854, 5,384,261, 5,424,186 5,445,934; and copending U.S. patent application Ser. No. 08/376,963, filed Jan. 23, 1995 (now issued as 5,959,298) incorporated herein by reference for all purposes.

The general methods of synthesizing oligomers on large arrays are known in the art. For example, U.S. Pat. No. 5,384,261 describes a method and device for forming large arrays of polymers on a substrate. According to a preferred aspect of the invention, the substrate is contacted by a channel block having channels therein. Selected reagents are flowed through the channels, the substrate is rotated by a rotating stage, and the process is repeated to form arrays of polymers on the substrate. The method may be combined with light-directed methodolgies.

The U.S. Pat. Nos. 5,143,854 and 5,424,186 describe methods for synthesizing polypeptide and oligonucleotide arrays. Polypeptide arrays can be synthesized on a substrate by attaching photoremovable protecting groups to the surface of a substrate, exposing selected regions of the substrate to light to activate those regions, attaching an amino acid monomer with a photoremovable group to the activated regions, and repeating the steps of activation and attachment until polypeptides of the desired length and sequences are synthesized.

The use of a photoremovable protecting group allows removal of selected portions of the substrate surface, via patterned irradiation, during the deprotection cycle of the solid phase synthesis. This selectively allows spatial control of the synthesis--the next amino acid is coupled only to the irradiated areas. The resulting array can be used to determine which peptides on the array can bind to a receptor.

The formation of oligonucleotides on a solid-phase support requires the stepwise attachment of a nucleotide to a substrate-bound growing oligomer. In order to prevent unwanted polymerization of the monomeric nucleotide under the reaction

conditions, protection of the 5'-hydroxyl group of the nucleotide is required. After the monomer is coupled to the end of the oligomer, the 5'-hydroxyl protecting group is removed, and another nucleotide is coupled to the chain. This cycle of coupling and deprotecting is continued for each nucleotide in the oligomer sequence. The use of a photoremovable protecting group allows removal, via patterned irradiation, of selected portions of the substrate surface during the deprotection cycle of the solid phase synthesis. This selectively allows spatial control of the synthesis-the next nucleotide is coupled only to the irradiated areas.

Preferably, the photosensitive protecting groups will be removable by radiation in the ultraviolet (UV) or visible portion of the electromagnetic spectrum. More preferably, the protecting groups will be removable by radiation in the near UV or visible portion of the spectrum. In some embodiments, however, activation may be performed by other methods such as localized heating, electron beam lithography, x-ray lithography, laser pumping, oxidation or reduction with microelectrodes, and the like. Sulfonyl compounds are suitable reactive groups for electron beam lithography. Oxidative or reductive removal is accomplished by exposure of the protecting group to an electric current source, preferably using microelectrodes directed to the predefined regions of the surface which are desired for activation. Other methods may be used in view of this disclosure.

When light is used to activate or deactivate various groups, the light may be from a conventional incandescent source, a laser, a laser diode, or the like. If non-collimated sources of light are used it may be desirable to provide a thick- or multi-layered mask to prevent spreading of the light onto the substrate. It may, further, be desirable in some embodiments to utilize groups which are sensitive to different wavelengths to control synthesis. For example, by using groups which are sensitive to different wavelengths, it is possible to select branch positions in the synthesis of a polymer or eliminate certain masking steps.

Note that different photoprotected monomers, such as amino acids, can exhibit different photolysis rates. It may be desirable to utilize photoprotected monomers with substantially similar photolysis rates in a particular application. To obtain such a set of photoprotected monomers, one merely needs to select the

appropriate photoprotecting group for each monomer in the set. In similar fashion, one can prepare a set of photoprotected monomers with substantially different photolysis rates (from monomer to monomer) by appropriate choice of photoprotecting groups.

Many, although not all, of the photoremovable protecting groups will be aromatic compounds that absorb near-UV and visible radiation. Suitable photoremovable protecting groups may be selected from a wide variety of positive light-reactive groups preferably including nitro aromatic compounds such as o-nitrobenzyl derivatives or benzylsulfonyl. In a preferred embodiment, 6-nitroveratryloxycarbonyl (NVOC), 2-nitrobenzyloxycarbonyl (NBOC) or .α,α-dimethyl-dimethoxybenzyloxycarbonyl (DDZ) is used. Additional examples of the photoremovable protecting groups include multiply substituted nitro aromatic compounds containing a benzylic hydrogen ortho to the nitro group, wherein the substituent may include alkoxy, alkyl, halo, aryl, alkenyl, nitro, halo, or hydrogen. Other materials which may be used include o-hydroxy-.alpha.-methyl cinnamoyl derivatives. Further examples of photoremovable protective groups may be found in, for example, Patchornik, *J. Am. Chem. Soc.* (1970) *92*:6333 and Amit *et al.*, *J. Org. Chem.* (1974) *39*:192.

The U.S. Pat. No. 5,413,854 notes that the positive reactive group may be activated for reaction with reagents in solution. For example, a 5-bromo-7-nitro indoline group, when bound to a carbonyl, undergoes reaction upon exposure to light at 420 nm. Alternatively, the reactive group on the linker molecule is selected from a wide variety of negative light-reactive groups including a cinammate group.

The U.S. Pat. No. 5,384,261 describes that the resulting substrate will have a variety of uses including, for example, screening large numbers of polymers for biological activity. To screen for biological activity, the substrate is exposed to one or more receptors such as an antibody whole cells, receptors on vesicles, lipids, or any one of a variety of other receptors. The receptors are preferably labeled with, for example, a fluorescent marker, such as fluorescein, radioactive marker, or a labeled antibody reactive with the receptor. In some cases, the channel block can be used to direct solutions containing a receptor over a synthesized array of polymers. For

example, the channel block is used to direct receptor solutions having different receptor concentrations over regions of the substrate.

The location of the marker on the substrate is detected with, for example, photon detection or autoradiographic techniques. Through knowledge of the sequence of the material at the location where binding is detected, it is possible to quickly determine which sequence binds with the receptor and, therefore, the technique can be used to screen large numbers of peptides. Amplification of the signal provided by way of fluorescein labeling is provided by exposing the substrate to the antibody of interest, and then exposing the substrate to a labeled material which is complementary to the antibody of interest and preferably binds at multiple locations of the antibody of interest. For example, if a mouse antibody is to be studied, a labeled second antibody may be exposed to the substrate which is, for example, goat antimouse.

Other possible applications of the inventions herein include diagnostics in which various antibodies for particular receptors would be placed on a substrate and, for example, blood sera would be screened for immune deficiencies. Still further applications include, for example, selective "doping" of organic materials in semiconductor devices, i.e., the introduction of selected impurities into the device and the like.

Examples of receptors which can be employed by this invention include, but are not restricted to, antibodies, cell membrane receptors, monoclonal antibodies and antisera reactive with specific antigenic determinants (such as on viruses, cells, or other materials), drugs, polynucleotides, nucleic acids, peptides, cofactors, lectins, sugars, polysaccharides, cells, cellular membranes, and organelles. Other examples of receptors include catalytic polypeptides, which are described in U.S. Pat. No. 5,215,899.

Thus, a related aspect of this invention provides a method of forming, from component molecules, a plurality of compounds on a support, each compound occupying a separate region of the support, said method comprising the steps of:

(a) activating a region of the support;

(b) binding a molecule to the region, said molecule comprising a masked

reactive site linked to a photolabile protecting group of the formula Y, and

(c) repeating steps (a) and (b) on other regions of the support whereby each of said other regions has bound thereto another molecule comprising a masked reactive site linked to the photolabile protecting group, wherein said another molecule may be the same or different from that used in step (b);

(d) removing the photolabile protecting group from one of the molecules bound to one of the regions of the support to provide a region bearing a molecule with an unmasked reactive site;

(e) binding an additional molecule to the molecule with an unmasked reactive site;

(f) repeating steps (d) and (e) on regions of the support until a desired plurality of compounds is formed from the component molecules, each compound occupying separate regions of the support.

A related method of forming a plurality of compounds on predefined regions of a support involves binding a molecule with a reactive site protected with a chemically labile protecting group to an activated region of the support and chemically removing the chemically labile protecting group to reveal the reactive site. The reactive site is then protected with a photolabile protecting group of this invention. This process is repeated for other regions of the support with other molecules as desired to provide a support having molecules with reactive sites protected by photolabile protecting groups on separate regions of the support. Reactive sites can be unmasked by removing the photolabile group from selected regions and coupled to additional molecules with photolabile protecting groups as described earlier to build up arrays of compounds on the support. Again, in a "mix and match" approach, monomers with chemically labile protecting groups can be attached to a reactive site on the substrate (i.e., on the support itself when the first layer of monomers is being assembled or subsequently onto an already attached monomer whose reactive site has been unmasked) and these chemically labile protecting groups can be replaced by a photolabile protecting groups of this invention. The replacement is accomplished by removing the chemically labile protecting group under conditions that do not affect any photolabile groups which

may be on the support. This then reveals an unmasked reactive site on the monomer which had carried the chemically labile protecting group and this unmasked reactive site is reacted with a reagent of the formula Y-X, where X is a leaving group. Thereby, this region of the support is protected by a photolabile protecting group which can be selectively removed by light directed systems described in U.S. Patent Nos. 5,143,854, 5,384,261, 5,424,186 and 5,445,934 and further described below (incorporated by reference in their entireties for all purposes). This method is particularly useful when the monomers are more readily available carrying chemically labile protecting groups than the photolabile protecting groups described herein. It will be recognized that any method of forming a chain of compounds or an array of compounds on a support using in at least one step a protecting group/reagent or compound of this invention is within the scope of the methods this invention.

Generally, these methods involve sequential addition of monomers to build up an array of polymeric species on a support by activating predefined regions of a substrate or solid support and then contacting the substrate with a protected monomer of this invention (*e.g.*, a protected nucleoside or amino acid). It will be recognized that the individual monomers can be varied from step to step. A common support is a glass or silica substrate as is used in semiconductor devices.

The predefined regions can be activated with a light source, typically shown through a screen such as a photolithographic mask similar to the techniques used in integrated circuit fabrication. Other regions of the support remain inactive because they are blocked by the mask from illumination and remain chemically protected. Thus, a light pattern defines which regions of the support react with a given monomer. The protected monomer reacts with the activated regions and is immobilized therein. The protecting group is removed by photolysis and washed off with unreacted monomer. By repeatedly activating different sets of predefined regions and contacting different monomer solutions with the substrate, a diverse array of polymers of known composition at defined regions of the substrate can be prepared. Arrays of 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12} or more different polymers can be assembled on the substrate. The regions may be 1 mm^2 or larger, typically 10 μm^2 and may be as small as 1 μm^2.

In the preferred methods of preparing these arrays, contrast between features may be enhanced through the front side exposure of the substrate. By "front side exposure" is meant that the activation light is incident upon the synthesis side of the substrate, contacting the synthesis side of the substrate prior to passing through the substrate. Front side exposure reduces effects of diffraction or divergence by allowing the mask to be placed closer to the synthesis surface. Additionally, and perhaps more importantly, refractive effects from the light passing through the substrate surface, prior to exposure of the synthesis surface, are also reduced or eliminated by front-side exposure. Front side exposure is described in substantial detail in U.S. patent application Ser. No. 08/634,053 filed Apr. 17, 1996 (now abandoned), incorprated herein by reference.

As noted previously, however, the efficiency of photolysis of the preferred photolabile protecting groups of the present invention is improved when such photolysis is carried out in the presence of nucleophilic solvents, such as water or methanol. This presents a unique problem where front side photolysis is used. Specifically, as the front side of the substrate is exposed to the activation radiation, a flow cell cannot be used to maintain the desired nucleophilic environment during such photolysis. Accordingly, in preferred aspects, light-directed synthesis methods employing the protecting groups of the present invention is carried out by providing a thin aqueous film or coating on the synthesis surface of the substrate. The presence of this thin film or coating allows one to control the local environment on the synthesis surface, i.e., to provide conditions that are favorable for that synthesis. By "conditions favorable to reaction" is meant conditions that result in an improvement of reaction efficiency of a given chemical reactant or reactants, over reactions not performed in that environment, e.g., reaction rate, yield, or both. For example, for synthesis methods employing the protecting groups described herein, coatings may be applied that provide a nucleophic environment which is favorable to photolysis of the protecting group, and which thereby promotes efficient synthesis. The use of such coatings also permits the front side exposure of the substrate surface. This method may also be performed in reacting more than one chemical reactant, by

applying both reactants on the surface prior to coating, or by adding the second reactant after the coating or as an element of the coating.

Generally, a thin film or coating of aqueous solution can be applied to the synthesis surface of a substrate that is bearing the protecting groups of the invention, e.g., that has been subjected to previous synthesis steps. Application of the coating may be carried out by methods that are well known in the art. For example, spin-coating methods may be utilized where the substrate is spun during application of the coating material to generate a uniform coating across the surface of the substrate. Alternative application methods may also be used, including simple immersion, spray coating methods and the like.

Aqueous solutions for use as coating materials typically include, e.g., low molecular weight poly-alcohols, such as ethylene glycol, propylene glycol, glycerol and the like. These solutions are generally hygrophilic and provide nucleophilic hydroxyl groups which will also support the photolysis reaction. The poly-alcohols also increase the viscosity of the solution, which can be used to control the thickness of the coating. Higher molecular weight poly-alcohols, i.e., polyvinyl alcohol, may also be used to adjust the viscosity of the coating material.

Generally, preferred substrates have relatively hydrophobic surfaces. As such, the aqueous coating solution may also include an appropriate surfactant, e.g., from about 0.01 to about 10% v/v to permit spreading and adhesion of the film upon the substrate surface. Such surfactants generally include those that are well known in the art, including, e.g., Triton X-100, Tween-80, and the like. In addition to promoting the spreading and adhesion of the coating to the substrate, addition of a these non-volatile solutes within the coating solution can limit the amount of evaporation of the film and promote its longevity.

The methods described herein may also employ component molecules comprising a masked reactive site attached to a photolabile protecting group having the structure Y. In such cases, the protecting group is attached to an acidic reactive site, such as a carboxylate or phophate and is removed by photolysis.

The solid substrate or solid support may be of any form, although they preferably will be planar and transparent (and potentially some three dimensional

structure). The supports need not necessarily be homogenous in size, shape or composition, although the supports usually and preferably will be uniform. In some embodiments, supports that are very uniform in size may be particularly preferred. In another embodiment, two or more distinctly different populations of solid supports may be used for certain purposes.

Solid supports may consist of many materials, limited primarily by capacity for derivatization to attach any of a number of chemically reactive groups and compatibility with the synthetic chemistry used to produce the array and, in some embodiments, the methods used for tag attachment and/or synthesis. Suitable support materials typically will be the type of material commonly used in peptide and polymer synthesis and include glass, latex, heavily cross-linked polystyrene or similar polymers, gold or other colloidal metal particles, and other materials known to those skilled in the art. The chemically reactive groups with which such solid supports may be derivatized are those commonly used for solid phase synthesis of the polymer and thus will be well known to those skilled in the art, *i.e.*, carboxyls, amines, and hydroxyls.

To improve washing efficiencies, one can employ nonporous supports or other solid supports less porous than typical peptide synthesis supports; however, for certain applications of the invention, quite porous beads, resins, or other supports work well and are often preferable. One such support is a resin in the form of beads. In general, the bead size is in the range of 1 nm to 100 μm, but a more massive solid support of up to 1 mm in size may sometimes be used. Particularly preferred resins include Sasrin resin (a polystyrene resin available from Bachem Bioscience, Switzerland); and TentaGel S AC, TentaGel PHB, or TentaGel S NH_2 resin (polystyrene-polyethylene glycol copolymer resins available from Rappe Polymere, Tubingen, Germany). Other preferred supports are commercially available and described by Novabiochem, La Jolla, California.

In other embodiments, the solid substrate is flat, or alternatively, may take on alternative surface configurations. For example, the solid substrate may contain raised or depressed regions on which synthesis takes place. In some embodiments, the solid substrate will be chosen to provide appropriate light-absorbing

characteristics. For example, the substrate may be a polymerized Langmuir Blodgett film, functionalized glass, Si, Ge, GaAs, GaP, SiO_2, SiN_4, modified silicon, or any one of a variety of gels or polymers such as (poly)tetrafluorethylene, (poly)vinylidendifluoride, polystyrene, polycarbonate, or combinations thereof. Other suitable solid substrate material will be readily apparent to those of skill in the art. Preferably, the surface of the solid substrate will contain reactive groups, which could be carboxyl, amino, hydroxyl, thiol, or the like. More preferably, the surface will be optically transparent and will have surface Si-OH functionalities, such as are found on silica surfaces.

The photolabile protecting groups and protected monomers disclosed herein can also be used in bead based methods of immobilization of arrays of molecules on solid supports.

A general approach for bead based synthesis is described in copending application Serial Nos. 07/762,522 (filed September 18, 1991); 07/946,239 (filed September 16, 1992); 08/146,886 (filed November 2, 1993); 07/876,792 (filed April 29, 1992) and PCT/US93/04145 (filed April 28, 1993), Lam et al. (1991) *Nature* 354:82-84; PCT application no. 92/00091 and Houghten et al, (1991) *Nature* 354:84-86, each of which is incorporated herein by reference for all purposes.

A single, planar solid support can be used to synthesize arrays of compounds, and the compounds can be cleaved from the support prior to screening using very large scale immobilized polymer synthesis (VLSIPS.TM.) technology. See U.S. Pat. No. 5,143,854, which is incorporated herein by reference. In one example, an array of oligonucleotides is synthesized on the VLSIPS.TM. chip, and each oligonucleotide is linked to the chip by a cleavable linker, such as a disulfide. See U.S. Pat. No. 5,412,087 (U.S. patent application Ser. No. 874,849, filed Apr. 24, 1992), incorporated herein by reference. The oligonucleotide tag has a free functional group, such as an amine, for attachment of the molecule to be tagged, which is typically an oligomer and preferably a peptide. The tag may optionally contain only pyrimidine or pyrimidine and purine analog bases. The tag also contains binding sites for amplification, i.e., PCR primer sites, optionally a sequencing primer site, and a short section uniquely coding the monomer sequence

of the oligomer to be tagged. Then, the oligomer is synthesized, i.e., from a free terminal amine groups on the tag or a linker linked to the tag, so that each oligomer is linked to a tag. The collection of tagged oligomers can be released from the chip by cleaving the linker, creating a soluble tagged oligomer library.

For bead-based syntheses, conventional techniques are used that are well-known in the art. For example, for the synthesis of peptides, Merrifield technique as described in Atherton et al., "Solid Phase Peptide Synthesis," IRL Press, (1989) will be used. Other synthesis techniques will be suitable when different monomers are used. For example, the techniques described in Gait *et al.*, *Oligonucleotide Synthesis*, will be used when the monomers to be added to the growing polymer chain are nucleotides. These techniques are only exemplary, and other more advanced techniques will be used in some embodiments such as those for reversed and cyclic polymer synthesis disclosed in U.S. Pat. No. 4,242,974.

It will be recognized that the monomers need not be directly coupled to the substrate, and linker molecules may be provided between the monomers and the substrate. Such linker molecules were described, for example, in the U.S. Pat. No. 5,445,934, at columns 11 and 12.

One can incorporate a wide variety of linkers, depending upon the application and effect desired. For instance, one can select linkers that impart hydrophobicity, hydrophilicity, or steric bulk to achieve desired effects on properties such as coupling or binding efficiency. In one aspect of the invention, branched linkers, i.e., linkers with bulky side chains such as the linker Fmoc-Thr(tBu), are used to provide rigidity to or to control spacing of the molecules on a solid support in a library or between a molecule and tag in the library.

Preferred photocleavable linkers include 6-nitroveratryloxycarbonyl (NVOC) and other NVOC related linker compounds. See U.S. Pat. No. 5,143,854 columns 11 through 13. In another embodiment, the linkers are nucleic acids with one or more restriction sites, so that one portion of a library member (either the tag, the oligomer or other compound of interest or both, or the solid support) can be selectively cleaved from another by the appropriate restriction enzyme. This novel nucleic acid linker

illustrates the wide variety of linkers that may be employed to useful effect for purposes of the present invention.

Synthetic oligodeoxyribonucleotides are especially preferred information-bearing identifier tags. Oligonucleotides are a natural, high density information storage medium. The identity of monomer type and the step of addition or any other information relevant to a chemical synthesis procedure is easily encoded in a short oligonucleotide sequence. Oligonucleotides, in turn, are readily amenable for attachment to a wide variety of solid supports, oligomers, linkers, and other molecules. For example, an oligonucleotide can readily be attached to a peptide synthesis bead.

The coupling steps for some of the monomer sets (amino acids, for example) can in some embodiments require a relatively lengthy incubation time, and for this and other reasons a system for performing many monomer additions in parallel is desirable. Automated instrumentation for use in generating and screening encoded synthetic molecular libraries, preferably those that are able to perform 50 to 100 or more parallel reactions simultaneously, is described in U.S. Pat. No. 5,503,805 (U.S. patent application Ser. No. 08/149,675, filed Nov. 2, 1993), incorporated herein by reference. Such an instrument is capable of distributing the reaction mixture or slurry of synthesis solid supports, under programmable control, to the various channels for pooling, mixing, and redistribution.

In general, however, the instrumentation for generating synthetic libraries of tagged molecules requires plumbing typical of peptide synthesizers, together with a large number of reservoirs for the diversity of monomers and the number of tags employed and the number of simultaneous coupling reactions desired. The tag dispensing capability translates simple instructions into the proper mixture of tags and dispenses that mixture. Monomer building blocks are dispensed, as desired, as specified mixtures. Reaction agitation, temperature, and time controls are provided. An appropriately designed instrument also serves as a multi-channel peptide synthesizer capable of producing 1 to 50 mgs (crude) of up to 100 specific peptides for assay purposes.

The invention as described herein applies to the preparation of molecules containing sequences of monomers such as amino acids as well as to the preparation of other polymers. Such polymers include, for example, both linear and cyclic polymers of nucleic acids, polysaccharides, phospholipids, and peptides having either .alpha.-, .beta.-, or .omega.-amino acids, heteropolymers in which a known drug is covalently bound to any of the above, polynucleotides, polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, polyacetates, or other polymers which will be apparent upon review of this disclosure. Such polymers are "diverse" when polymers having different monomer sequences are formed at different predefined regions of a substrate.

In addition, the invention can readily be applied to the preparation of any set of compounds that can be synthesized in a component-by-component fashion, as can be appreciated by those skilled in the art. For instance, compounds such as benzodiazepines, hydantoins, and peptidylphosphonates can be prepared using the present methods. See U.S. Pat. No. 5,420,328, which is incorporated by reference. Methods of cyclization and polymer reversal of polymers which may be used in conjunction with the present invention are disclosed in U.S. Pat. No. 5,242,974, incorporated herein by reference.

Other methods of immobilization of arrays of molecules in which the photocleavable protecting groups of this invention can be used include pin based arrays and flow channel and spotting methods.

Photocleavable arrays also can be prepared using the pin approach developed by Geysen et al. for combinatorial solid-phase peptide synthesis. A description of this method is offered by Geysen et al., *J. Immunol. Meth.* (1987) 102:259-274, incorporated herein by reference.

Additional methods applicable to library synthesis on a single substrate are described in U.S. Patent Nos. 5,384,261, 5,677,195, 6,040,193 that are hereby incorporated by reference in their entireties for all purposes. In the methods disclosed in these applications, reagents are delivered to the substrate by either (1) flowing within a channel defined on predefined regions or (2) "spotting" on

predefined regions. However, other approaches, as well as combinations of spotting and flowing, may be employed. In each instance, certain activated regions of the substrate are mechanically separated from other regions when the monomer solutions are delivered to the various reaction sites. Photocleavable linkers are particularly suitable for this technology as this delivery method may otherwise result in poor synthesis fidelity due to spreading, reagent dilution, inaccurate delivery, and the like. By using a photocleavable linker, rather than a conventional acid-cleavable linker, the purest material can be selectively cleaved from the surface for subsequent assaying or other procedures. More specifically, masks can be used when cleaving the linker to ensure that only linker in the center of the delivery area (*i.e.*, the area where reagent delivery is most consistent and reproducible) is cleaved. Accordingly, the material thus selectively cleaved will be of higher purity than if the material were taken from the entire surface.

Typically, the molecules used in this method will be the monomeric components of complex macromolecules. These monomeric components can be small ligand molecules, amino acids, nucleic acids, nucleotides, nucleosides, monosaccharides and the like, thereby allowing one to synthesize arrays of complex macromolecules or polymeric sequences, such as polypeptides, nucleic acids and synthetic receptors, on the solid support.

EXAMPLES

I. Synthetic Methods

Examples of the preferred groups shown in Figure 2 were synthesized and tested as 5'-photolabile protecting groups on thymidine phosporamidite monomers. Surface photolysis rates in different solvents (std. 365nm lightsource) were determined as described elsewhere (McGall et al., JACS 1997, 119: 5081, hereby incorporated by reference in its entirety for all purposes). Standard coupling efficiency measurements were made using the cleavable linker HPLC analysis technique (see U.S.S.No. 09/545,207, and attorney docket no. 3233.1, which are both hereby incorporated by reference in their entireties).

Figure 1 shows the preferred compounds and their synthesis. It shows the general structures of the preferred structures, the preferred structures, their synthesis, the yields of the nucleic acid sequences formed using the preferred protecting groups, and the photolysis conditions. Also, the synthesis steps are annotated with references that relate to the specific synthesis. All of these references are hereby incorporated by reference in their entireties for all purposes.

5'-TEMPOC-T-Phosphoramidite was synthesized using the steps outlined in Fig. 3 and the details shown in the references in that Figure. Specifically, the following references are hereby incorporated by reference in their entireties for all purposes as well as the steps that are cited: Dyer, et al. JOC 64:7988 (1999); Tetrahedron Lett., 38(52), 8933-4 (1997); Mcgall, et al., JACS 119:5081 (1997). The Fig. indicates that triphosgene may work equally well for step #1 and that chloroformate could probably be used without purification in step #2. NINOC-T-CEP was synthesized according to the steps shown in Fig. 4 and the following references are incorporated by reference in their entireties for all purposes as well as the steps that are cited; Bromidge, et al. (1998) J. Med. Chem. 41: 1598; Brooker, LS, et al. (1953) U.S. Patent No. 2,646,430; Boekelheide, et al. (1954) J. Org. Chem. 19:.504; Bennet, et al. (1941) J. Chem. Soc. 74:244; and Mortensen, et al. (1996) Org. Prep. Proc. Int. 28: 123. Figs. 5-8 show the synthesis of the following compounds; Me2NPOC-T-CEP; Me3NPOC-T-CEP; and NA1BOC-T-CEP. Fig. 8 refers to Aust. J. Chem 48:1969-70 which is also incorporated by reference in its entirety. Abbreviations used in the first step of the processes indicate the source of the material. For example, DAV is Davos, LAN is Lancaster, ALH is Adrich. CEP stands for cyanoethyl N, N diisopropyl phosphoramidite.

Figures 9 through 20 provide method for synthesizing other compounds of the invention.

II. Photolysis Studies

Surface photolysis rates and stepwise synthesis efficiency (or cycle yield) were carried out following the method described in McGall, *et al.*, *J. Am. Chem. Soc.* (1997), *119*(22):5081, the entire teachings of which are incorporated

herein by reference. The half-life for cleavage of protecting groups of the invention and cycle yield under various photolysis conditions are listed in Table 1.

Table 1: Photolysis Studies

Photolabile Protecting Group	***Photolysis Conditions***	***Photospeed (half-lives/Joule)***	***Cycle Yield (%)***
MeNPOC	methanol:water	0.9	88
MeNPOC	dry	1.9	83
MeNPTEOC	methanol:water	3.6	25
BNIC	2% NMI/DMSO	1.1	72
NIC	2%NMI/DMSO	3.1	92
MNAC	methanol:water	0.1	94
MNPOC-4	methanol:water	4.3	70
MNPOC-6	dioxane:water	1.1	32
NPPOC	2%NMI/DMSO	1.5	94
MNPPOC-45	2%NMI/DMSO	2.9	89
NNEOC-81	2%NMI/DMSO	3.4	94
NNEOC-21	dioxane	0.7	75
Bis MeNPOC	methanol	2.2	92
Bis NVOC	Dioxane	3.1	94

74	DEACMOC-	dry /	20	96
	DMCMOC-674	methanol	1.06	not evaluated

The foregoing invention has been described in some detail by way of illustration and examples, for purposes of clarity and understanding. It will be obvious to one of skill in the art that changes and modifications may be practiced within the scope of the appended claims. Therefore, it is to be understood that the above description is intended to be illustrative and not restrictive. The scope of the invention should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the following appended claims, along with the full scope of equivalents to which such claims are entitled.

All patents, patent applications and publications cited in this application are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual patent, patent application or publication were so individually denoted.

CLAIMS

What is claimed is:

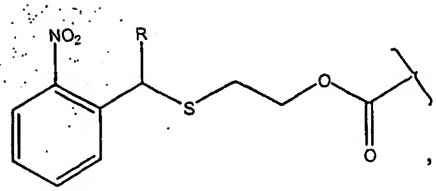
1. A compound represented by the following structural formula:

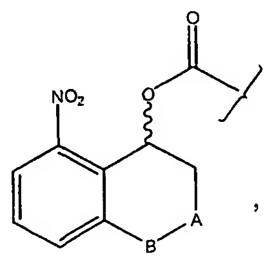
Y-X

wherein:

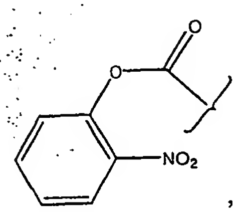
X is a leaving group or a compound having a masked reactive site; and

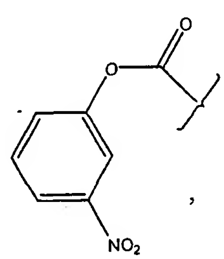
Y is a photolabile protecting group selected from the group consisting of:

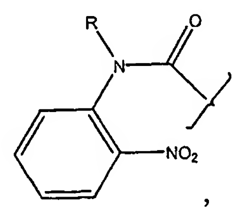


,


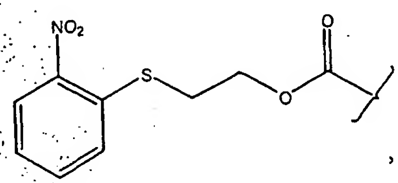
,

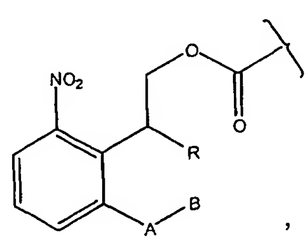


,


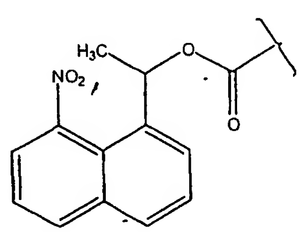
,


,



,


,

or


wherein:

R is -H, an optionally substituted alkyl, or an optionally substituted aryl;

A is -O-, -S-, -NR-, or -$(CH_2)_k$-;

k is 0 or an integer from one to about three; and

B is a monovalent or divalent aprotic weakly basic group.

2. The compound of Claim 1, wherein X is a compound having a masked reactive site and X further comprises a reactive site.

3. The compound of Claim 2, X is selected from the group consisting of an amino acid, a peptide, nucleoside, nucleotide, polynucleotide or analogs thereof, a monosaccharide and a protein.

4. The compound of Claim 2, wherein X is a base-protected deoxynucleoside, wherein the deoxynucleoside is a deoxyadenosine, a deoxycytidine, a thymidine or a deoxyguanosine.

5. The compound of Claim 4, wherein X is selected from the group consisting of base protected deoxynucleoside H-phosphonates and base protected deoxynucleoside phosphoramidites.

6. A method of attaching a molecule with a reactive site to a support comprising the steps of:

(a) providing a support with a reactive site;

(b) reacting the reactive site of a first compound of Claim 2 with the support to form a bond; and

(c) removing the photolabile protecting group to provide a derivatized support comprising the compound of Claim 2 with an unmasked reactive site immobilized thereon.

7. The method of Claim 6, wherein a covalent bond is formed in step (b).

8. The method of Claim 6, further comprising:
(d) reacting the reactive site of a second compound of Claim 2 with the unmasked reactive site of the first compound to form a bond; and
(e) removing the photolabile protecting group of the second compound to provide a support derivatized with a dimer chain having an unmasked reactive site immobilized thereon.

9. The method of Claim 8, further comprising repeating steps (d) and (e) with a succession of compounds to provide a chain of molecules immobilized on the support.

10. The method of Claim 9, wherein the molecules are deoxynucleosides.

11. The method of Claim 6, wherein the support is a glass or silica substrate.

12. The method of Claim 9, wherein the deoxynucleosides are linked to the photolabile group via a 5'-OH.

13. The method of Claim 8, wherein the photolabile group is removed by irradiation at a wavelength of greater than 350 nm.

14. The method of Claim 13, wherein the wavelength is about 365 nm.

15. A method of forming, from component molecules, a plurality of support bound compounds, each compound occupying a separate predefined region of the support, said method comprising the steps of:
(a) activating a first predefined region of a support;
(b) binding a molecule to the first region, wherein said molecule is a compound of Claim 2;

(c) repeating steps (a) and (b) on other predefined regions of the support whereby each of said other regions has bound thereto another molecule, wherein said another molecule is a compound of Claim 2, and wherein said another molecules may be the same or different from that used in step (b);

(d) removing the photolabile protecting group from molecules bound to one of the regions of the support to provide a region bearing molecules with an unmasked reactive site;

(e) binding an additional molecule to the molecule with an unmasked reactive site, wherein the additional molecule is a compound of Claim 2;

(f) repeating steps (d) and (e) on regions of the support until a plurality of support bound compounds is formed from the component molecules, each compound occupying separate regions of the support.

16. The method of Claim 15, wherein a covalent bond is formed in steps (b) and (e).

17. The method of Claim 15, wherein the molecules are deoxynucleosides.

18. The method of Claim 15, wherein the support is a glass or silica substrate.

19. The method of Claim 17, wherein the deoxynucleosides are linked to the photolabile group via a 5'-OH or the 3'-OH.

20. The method of Claim 15, wherein the photolabile group is removed by irradiation at a wavelength of greater than 350 nm.

21. The method of Claim 20, wherein the wavelength is about 365 nm.

22. The method of Claim 15, wherein at least 10^6 chains are immobilized on the support.

23. The method of Claim 15, wherein each of the regions has an area of between about 1 μm^2 and 10,000 μm^2.

24. The method of Claim 15, further comprising:

(g) covalently binding a second molecule comprising a masked reactive site linked to a chemically labile protecting group to a reactive site, wherein the reactive site is either on an activated region of the support as formed in step (a) or is an unmasked reactive site on a molecule on the support as formed in step (d);

(h) cleaving the chemically labile protecting group to form an unmasked reactive site;

(i) reacting a molecule of Claim 1 or 2 with the unmasked reactive site, thereby replacing the chemically labile protecting group with the photolabile protecting group to provide a region of the support having a molecule with the photolabile protecting group; and

(j) optionally repeating steps (d) - (f).

25. A compound as recited in claim 1 wherein the compound Y is Me2NPOC; Me3NPOC; NP2NPOC; NA1BOC; 5'-TEMPOC and NINOC.

26. A compound as recited in claim 5 wherein the compound Y is Me2NPOC-T-CEP; Me3NPOC-T-CEP; NP2NPOC-T-CEP; NA1BOC-T-CEP; 5'-TEMPOC-T-Phosporamidite, and NINOC-T-CEP.

27. A method in accordance with claim 10 wherein the compound Y is Me2NPOC; Me3NPOC; NP2NPOC; NA1BOC; 5'-TEMPOC, and NINOC.

28. A method in accordance with claim 9 wherein the compound Y is ME2NPOC-T-CEP; Me3NPOC-T-CEP; NP2NPOC-T-CEP; NA1BOC-T-CEP; 5'-TEMPOC-T-Phosporamidite.

29. A method in accordance with claim 14 wherein the compound Y is Me2NPOC; Me3NPOC; NP2NPOC; NA1BOC; 5'-TEMPOC, and NINOC.

30. A method in accordance with claim 16 wherein the compound Y is Me2NPOC-T-CEP; Me3NPOC-T-CEP; NP2NPOC-T-CEP; NA1BOC-T-CEP; 5'-TEMPOC-T-Phosporamidite and NINOC-T-CEP.

31. A compound represented by the following structural formula:

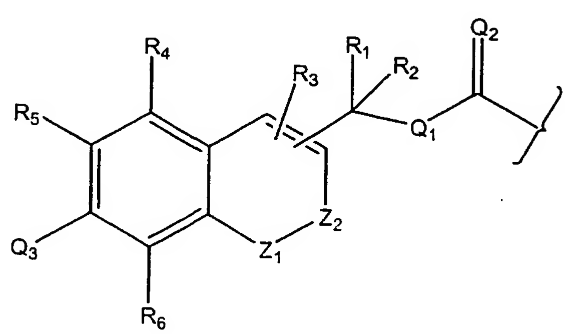
Y-X

wherein:

X is a leaving group or a compound having a masked reactive site;

and

Y is a photolabile protecting group bound to the leaving group or masking the masked reactive site, wherein Y is represented by the following structural formula:



wherein:

R_1 and R_2 are each, independently, -H, an optionally substituted alkyl,

an optionally substituted alkenyl, an optionally substituted alkynyl, a trialkylsilyl, an optionally substituted aryl, an optionally substituted heteroaryl or a vinylogous derivative of the foregoing groups;

Q_1 is –O-, -S-, -CH_2O- or –CH_2S-;

Q_2 is =O or =S

R_3 and R_4 are each, independently, -H, an optionally substituted alkyl, an optionally substituted aryl, an optionally substituted alkoxy, or –NO_2, provided that when one of R_3 or R_4 is –NO_2, at least one of R_1 or R_2 is –H;

R_5 and R_6 are each, independently, -H, an optionally substituted alkyl, an optionally substituted aryl, or an optionally substituted alkoxy;

Q_3 is –H, an optionally substituted alkoxy, or a dialkylamino;

Z_1 and Z_2 taken together are –OC(O)-, -$NR_7C(O)$-, or –CR_8=CR_9-;

R_7 is –H or an alkyl;

R_8 is –H, an optionally substituted alkyl, an optionally substituted aryl, or an optionally substituted alkoxy; and

R_9 is –H, an optionally substituted alkyl, an optionally substituted aryl, or an optionally substituted alkoxy or –NO_2; or

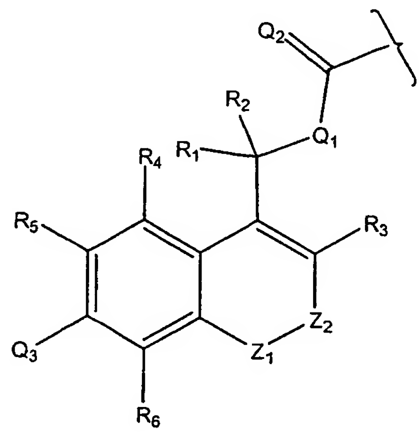
R_8 and R_9, together with the carbon atoms to which they are attached, form a five or six membered carbocyclic or heterocyclic ring, provided that when none of R_3, R_4 or R_9 are –NO_2, Q_1 is not –CH_2O- or –CH_2S-.

32. The compound of Claim 31, wherein X is a compound having a masked reactive site and X further comprises a reactive site.

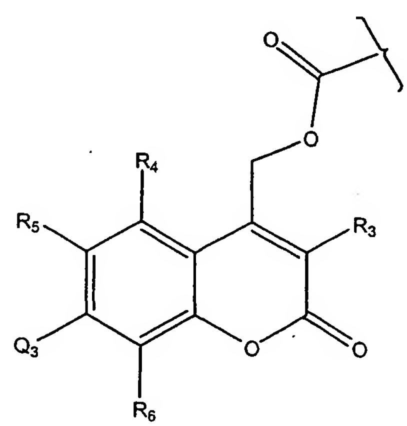
33. The compound of Claim 32, wherein X is a compound having a masked reactive site selected from the group consisting of an amino acid, a nucleoside, a nucleoside phosphoramidite, a nucleoside H-phosphonate, a nucleotide, a solid support, a peptide, an oligonucleotide, a protein, a hormone, an antibody, a polysaccharide, a monosaccharide, a disaccharide, a solid support bound peptide, a solid support bound oligonucleotide, a solid support bound protein, a solid support bound hormone, a solid support bound

antibody, a solid support bound polysaccharide, a solid support bound monosaccharide, or a solid support bound disaccharide.

34. The compound of Claim 31, wherein Y is represented by the following structural formula:



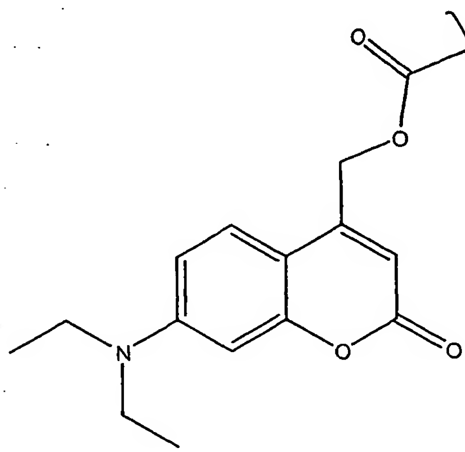
35. The compound of Claim 34, wherein the Y is represented by the following structural formula:



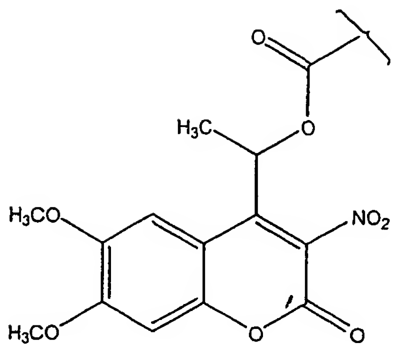
36. The compound of Claim 35, wherein one of R_3 or R_4 is $-NO_2$.

37. The compound of Claim 35, wherein R_3, R_4, R_5 and R_6 are –H and Q_3 is a dialkylamino.

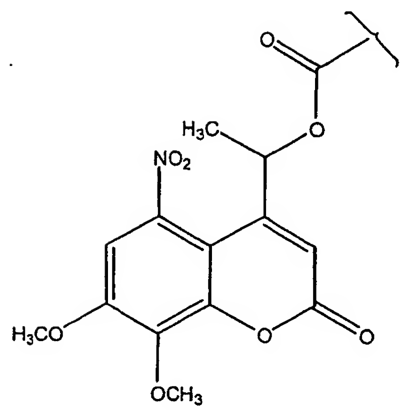
38. The compound of Claim 36, wherein Y is represented by the following structural formula:



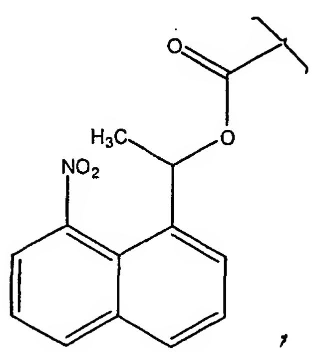
39. The compound of Claim 34, wherein Y is selected from the group consisting of:



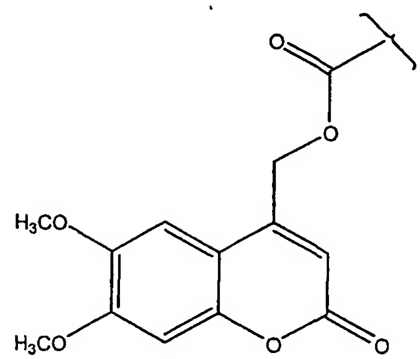
,



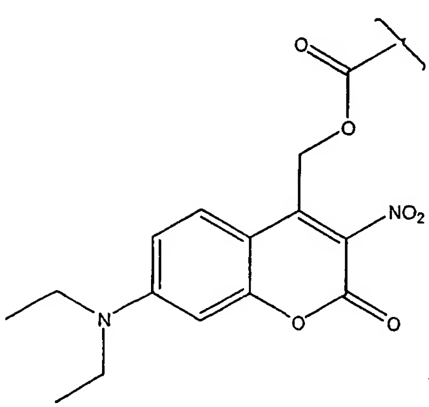
,



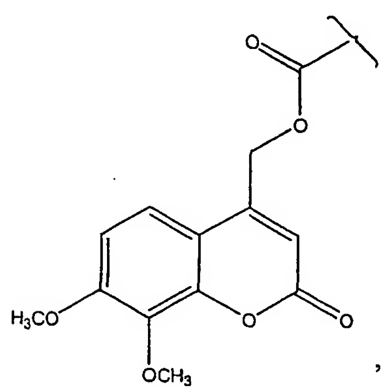
,



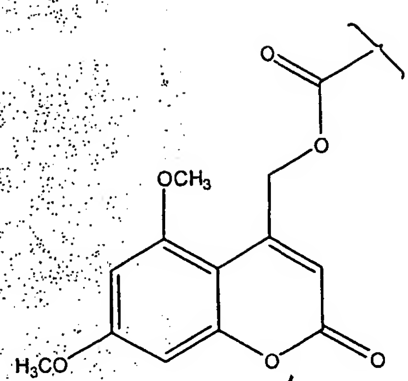
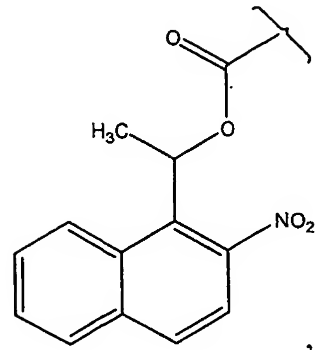
,

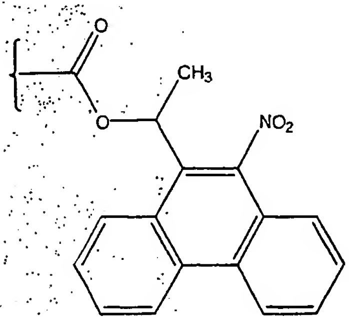
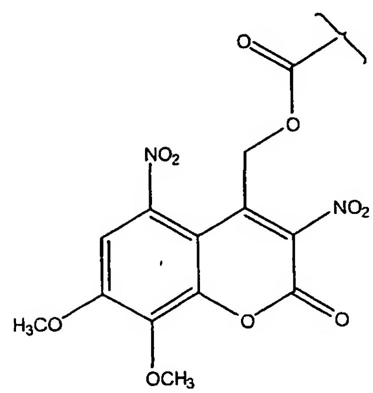


,

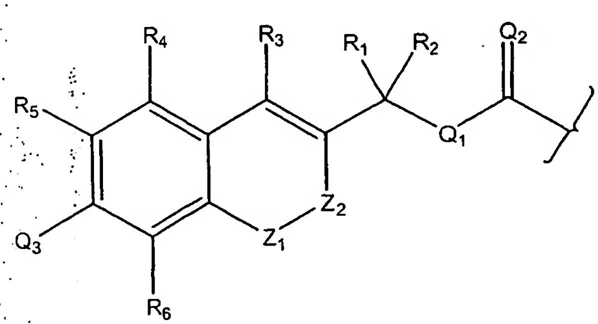


,

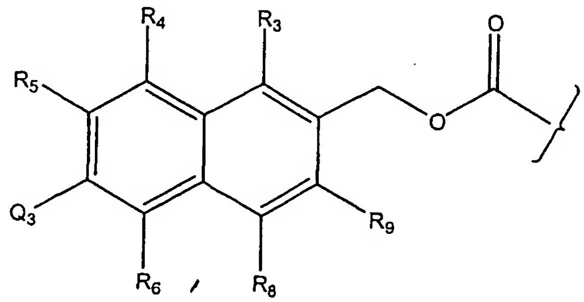

,

,


, and

.

40. The compound of Claim 31, wherein Y is a group represented by the following structural formula:

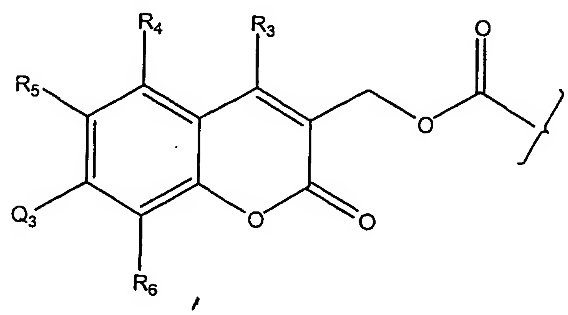


41. The compound of Claim 40, wherein Y is represented by the following structural formula:



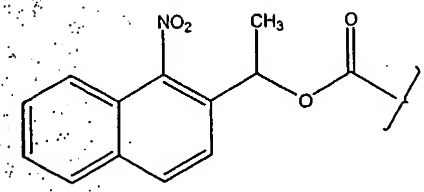
42. The compound of Claim 41, wherein one of R_3 or R_9 is $-NO_2$.

43. The compound of Claim 40, wherein Y is represented by the following structural formula:

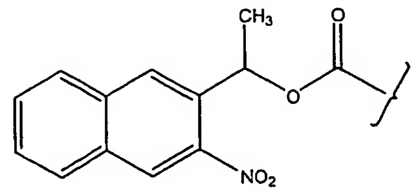


44. The compound of Claim 43, wherein R_3, R_4, R_5 and R_6 are –H and Q_3 is a dialkylamino.

45. The compound of Claim 40, wherein Y is selected from the group consisting of:

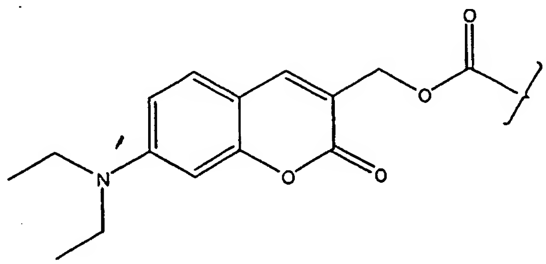


,



,

and



46. A compound represented by the following structural formula:

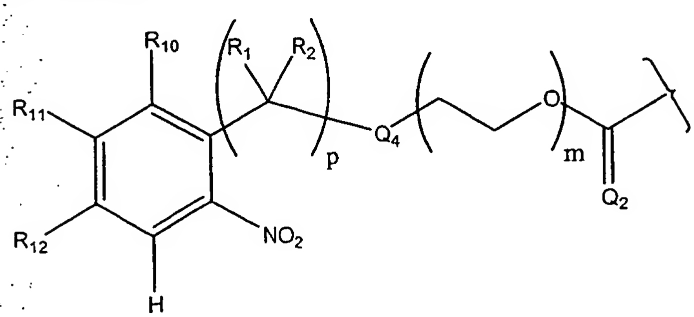
Y-X

wherein:

X is a leaving group or a compound having a masked reactive site;

and

Y is a photolabile protecting group bound to the leaving group or masking the masked reactive site, wherein Y is represented by the following structural formula:



wherein:

m is 0 or 1;

p is 0, 1 or 2;

R_1 and R_2 for each occurrence are, independently, -H, an optionally substituted alkyl, an optionally substituted alkenyl, an optionally substituted alkynyl, a trialkylsilyl, an optionally substituted aryl, an optionally substituted heteroaryl or a vinylogous derivative of the foregoing groups;

Q_2 is =O or =S;

Q_4 is –O-, -S-, or $-NR_{13}-$;

R_{13} is –H, an optionally substituted alkyl or an optionally substituted aryl;

R_{10} is –H, an optionally substituted alkyl, an optionally substituted aryl, an optionally substituted alkoxy or $-NO_2$; or

R_{10} and R_{13} together with the carbon atom and nitrogen atom to which they are form a five or six membered heterocycle; and

R_{11} and R_{12} are each, independently, -H, a halogen, an optionally substituted alkyl, an optionally substituted aryl, or an optionally substituted alkoxy; or

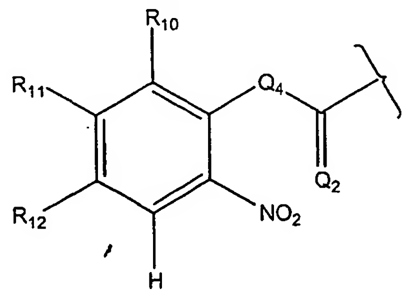
R_{11} and R_{12} taken together with the carbons to which they are attached form a five or six membered carbocycle or heterocycle.

47. The compound of Claim 46, wherein X is a compound having a masked reactive site and X further comprises a reactive site.

48. The compound of Claim 47, wherein X is a compound having a masked reactive site selected from the group consisting of an amino acid, a nucleoside, a nucleoside phosphoramidite, a nucleoside H-phosphonate, a nucleotide, a solid support, a peptide, an oligonucleotide, a protein, a hormone, an antibody, a polysaccharide, a monosaccharide, a disaccharide, a solid support bound peptide, a solid support bound oligonucleotide, a solid support bound protein, a solid support bound hormone, a solid support bound

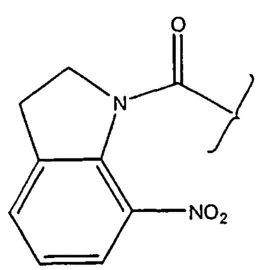
antibody, a solid support bound polysaccharide, a solid support bound monosaccharide, or a solid support bound disaccharide.

49. The compound of Claim 46, wherein m and p are both 0 and Y is represented by the following structural formula:

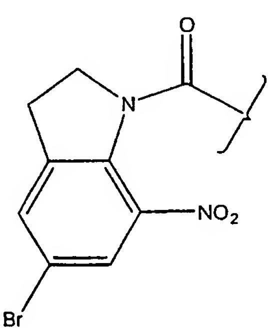


,

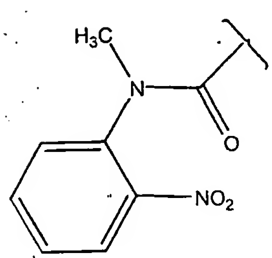
50. The compound of Claim 49, wherein Y is selected from the group consisting of:



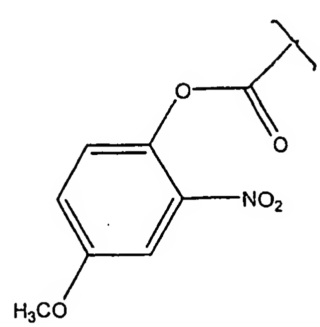
,



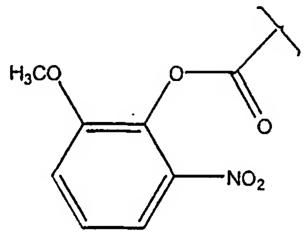
,

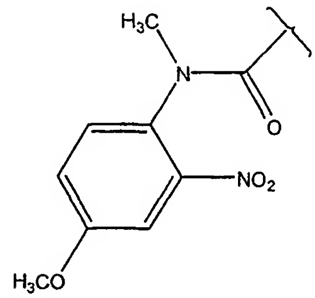


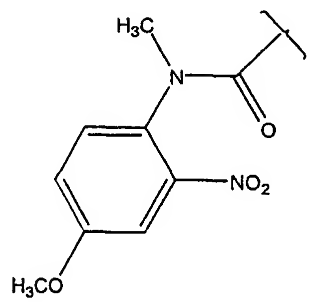
,



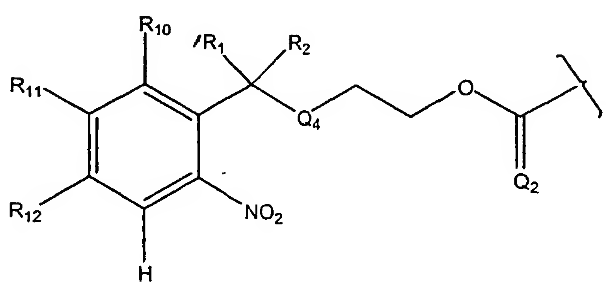
,

,

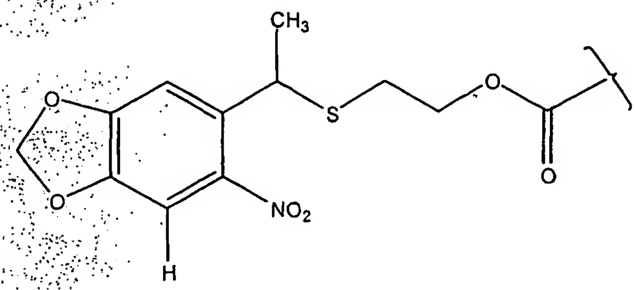
,

and .

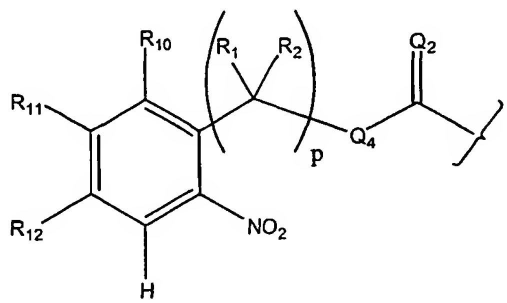
51. The compound of Claim 46, wherein m is 1 and p is 1 and Y is represented by the following structural formula:



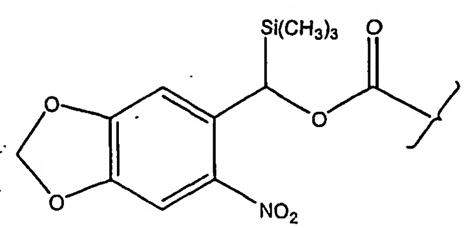
52. The compound of Claim 51, wherein Y is represented by the following structural formula:



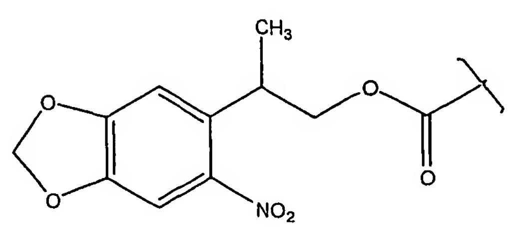
53. The compound of Claim 46, wherein m is 0 and p is 1 or 2, and Y is represented by the following structural formula:



54. The compound of Claim 53, wherein Y is selected from the group consisting of:



and



55. A method of attaching a molecule with a reactive site to a support comprising the steps of:
    (a) providing a support with a reactive site;
    (b) reacting the reactive site of a first compound of Claim 32 with the support to form a bond; and
    (c) removing the photolabile protecting group to provide a derivatized support comprising the compound of Claim 32 with an unmasked reactive site immobilized thereon.

56. The method of Claim 55, wherein the bond formed in step (b) is covalent.

57. The method of Claim 55, further comprising the steps of:
    (d) reacting the reactive site of a second compound of Claim 32 with the unmasked reactive site of the first compound to form a bond; and
    (e) removing the photolabile protecting group of the second compound to provide a support derivatized with a dimer chain having an unmasked reactive site immobilized thereon; and
    (f) optionally repeating steps (d) and (e) with a succession of molecules to provide an oligomer immobilized on the support.

58. The method of Claim 57, wherein the molecules are deoxynucleosides.

59. The method of Claim 57, wherein the support is a glass or silica substrate.

60. The method of Claim 58, wherein the deoxynucleosides are linked to the photolabile group via a 5'-OH.

61. The method of Claim 57, wherein the photolabile group is removed by irradiation at a wavelength of greater than 350 nm.

62. The method of Claim 61, wherein the wavelength is about 365 nm.

63. A method of forming, from component molecules, a plurality of support bound compounds, each compound occupying a separate predefined region of the support, said method comprising the steps of:

(a) activating a region of the support;

(b) binding a molecule to the first region, wherein said molecule is a compound of Claim 32;

(c) repeating steps (a) and (b) on other regions of the support whereby each of said other regions has bound thereto another molecule, wherein said another molecule is a compound of Claim 32, and wherein said another molecules may be the same or different from that used in step (b);

(d) removing the photolabile protecting group from molecules bound to one of the regions of the support to provide a region bearing molecules with an unmasked reactive site;

(e) binding an additional molecule to the molecule with an unmasked reactive site, wherein the additional molecule is a compound of Claim 32;

(f) repeating steps (d) and (e) on regions of the support until a plurality of support bound compounds is formed from the component molecules, each compound occupying separate regions of the support.

64. The method of Claim 63, wherein a covalent bond is formed in steps (b) and (e).

65. The method of Claim 63, wherein the molecules are deoxynucleosides.

66. The method of Claim 63, wherein the support is a glass or silica substrate.

67. The method of Claim 65, wherein the deoxynucleosides are linked to the photolabile group via a 5'-OH or a 3'-OH.

68. The method of Claim 63, wherein the photolabile group is removed by irradiation at a wavelength of greater than 350 nm.

69. The method of Claim 68, wherein the wavelength is about 365 nm.

70. The method of Claim 63, wherein at least 10^6 compounds are immobilized on the support.

71. The method of Claim 63, wherein each of the regions has an area of between about 1 μm^2 and 10,000 μm^2.

72. The method of Claim 63, further comprising:

(g) covalently binding a second molecule comprising a masked reactive site linked to a chemically labile protecting group to a reactive site, wherein the reactive site is either on an activated region of the support as formed in step (a) or is an unmasked reactive site on a molecule on the support as formed in step (d);

(h) cleaving the chemically labile protecting group to form an unmasked reactive site;

(i) reacting a molecule of Claim 32 with the unmasked reactive site, thereby replacing the chemically labile protecting group with the photolabile protecting group to provide a region of the support having a molecule with the photolabile protecting group; and

(j) optionally repeating steps (d) - (f).

73. A method of attaching a molecule with a reactive site to a support comprising the steps of:

(a) providing a support with a reactive site;

(b) reacting the reactive site of a first compound of Claim 47 with the support to form a bond; and

(c) removing the photolabile protecting group to provide a derivatized support comprising the compound of Claim 47 with an unmasked reactive site immobilized thereon.

74. The method of Claim 73, wherein the bond formed in step (b) is covalent.

75. The method of Claim 73, further comprising the steps of:

(d) reacting the reactive site of a second compound of Claim 47 with the unmasked reactive site of the first compound to form a bond; and

(e) removing the photolabile protecting group of the second compound to provide a support derivatized with a dimer chain having an unmasked reactive site immobilized thereon; and

(f) optionally repeating steps (d) and (e) with a succession of molecules to provide an oligomer immobilized on the support.

76. The method of Claim 75, wherein the molecules are deoxynucleosides.

77. The method of Claim 75, wherein the support is a glass or silica substrate.

78. The method of Claim 76, wherein the deoxynucleosides are linked to the photolabile group via a 5'-OH.

79. The method of Claim 75, wherein the photolabile group is removed by irradiation at a wavelength of greater than 350 nm.

80. The method of Claim 79, wherein the wavelength is about 365 nm.

81. A method of forming, from component molecules, a plurality of support bound compounds, each compound occupying a separate predefined region of the support, said method comprising the steps of:

(a) activating a region of the support;

(b) binding a molecule to the first region, wherein said molecule is a compound of Claim 47;

(c) repeating steps (a) and (b) on other regions of the support whereby each of said other regions has bound thereto another molecule, wherein said another molecule is a compound of Claim 47, and wherein said another molecules may be the same or different from that used in step (b);

(d) removing the photolabile protecting group from molecules bound to one of the regions of the support to provide a region bearing molecules with an unmasked reactive site;

(e) binding an additional molecule to the molecule with an unmasked reactive site, wherein the additional molecule is a compound of Claim 47;

(f) repeating steps (d) and (e) on regions of the support until a plurality of support bound compounds is formed from the component molecules, each compound occupying separate regions of the support.

82. The method of Claim 81, wherein a covalent bond is formed in steps (b) and (e).

83. The method of Claim 81, wherein the molecules are deoxynucleosides.

84. The method of Claim 81, wherein the support is a glass or silica substrate.

85. The method of Claim 83, wherein the deoxynucleosides are linked to the photolabile group via a 5'-OH or a 3'-OH.

86. The method of Claim 81, wherein the photolabile group is removed by irradiation at a wavelength of greater than 350 nm.

87. The method of Claim 86, wherein the wavelength is about 365 nm.

88. The method of Claim 81, wherein at least 10^6 compounds are immobilized on the support.

89. The method of Claim 81, wherein each of the regions has an area of between about 1 μm^2 and 10,000 μm^2.

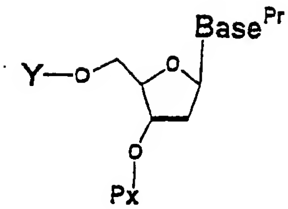
90. The method of Claim 81, further comprising:

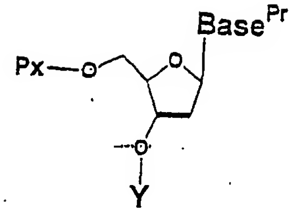
(g) covalently binding a second molecule comprising a masked reactive site linked to a chemically labile protecting group to a reactive site, wherein the reactive site is either on an activated region of the support as formed in step (a) or is an unmasked reactive site on a molecule on the support as formed in step (d);

(h) cleaving the chemically labile protecting group to form an unmasked reactive site;

(i) reacting a molecule of Claim 47 with the unmasked reactive site, thereby replacing the chemically labile protecting group with the photolabile protecting group to provide a region of the support having a molecule with the photolabile protecting group; and

(j) optionally repeating steps (d) - (f).

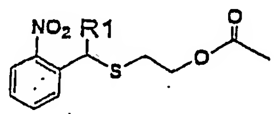




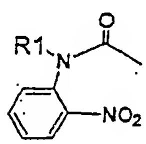
Px = phosphoramidite, H-phosphonate, phosphate triester

Y = general structures (R1 = H, alkyl, aryl)

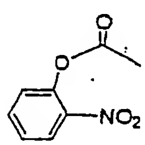
o-nitrobenzylthioethyloxycarbonyl NBTEOC:



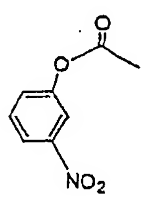
o-nitrophenylaminocarbonyl NPAC



o-nitrophenoxycarbonyl N2POC



m-nitrophenoxycarbonyl N3POC



o-nitrophenylthioethyloxycarbonyl

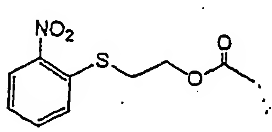
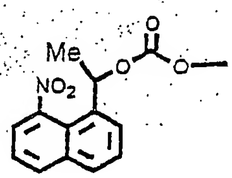
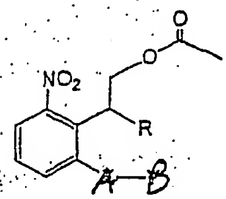


Figure 1A

α-methyl-8-nitronaphthylmethoxycarbonyl MeNMOC



6-substituted 2-(o-nitrophenyl)-2-propyloxycarbonyl 6NPPOC



(A = O, S, N-alkyl, N-aryl, (CH2)n, where n = 0 - ~3)
(B = aprotic weakly basic group - eg.: N-alkylimidazole)

cyclic o-nitrobenzyloxycarbonyl

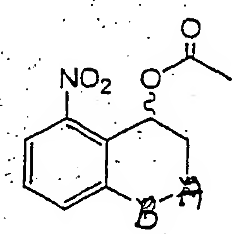
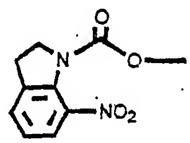
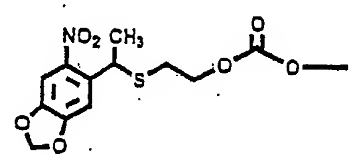


Figure 1B

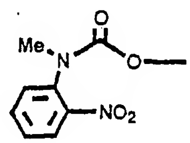
NIOC



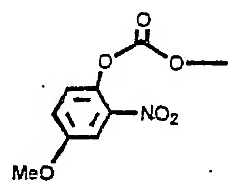
TEMPOC



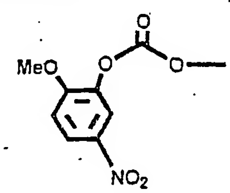
NAMOC



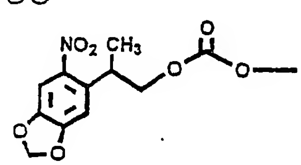
MeN2POC



MeN3POC



NP2POC



NNEOC

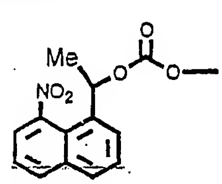


Figure 2A

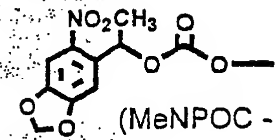
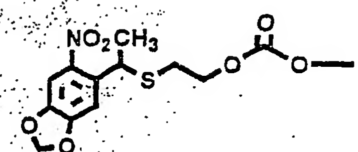
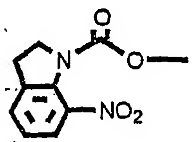
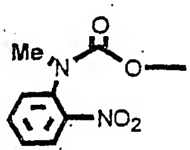
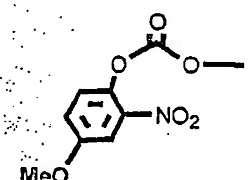
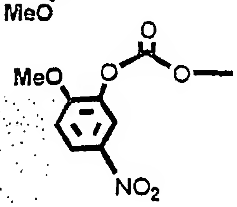
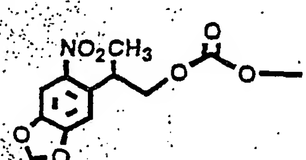
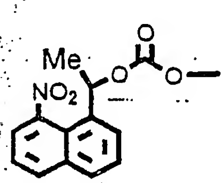
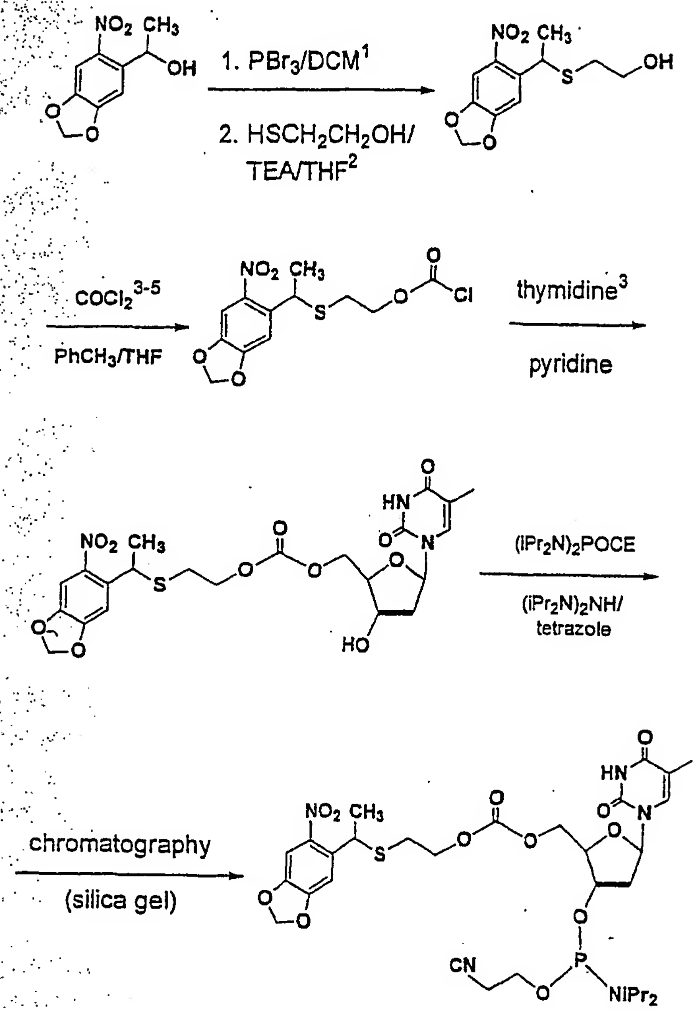
Y =	stepwise yield	photolysis conditions
 (MeNPOC - control)	~88 %	nonpolar solvent
	~85 %	MeOH
	95%	DMSO
	94%	nucleophilic solvent (MeOH)
	~80%	nucleophilic solvent (MeOH)
	~75 %	nucleophilic solvent (MeOH)
	90 %	basic solvent (1%NMI/DMSO)
	96 %	DMSO

Figure 2B

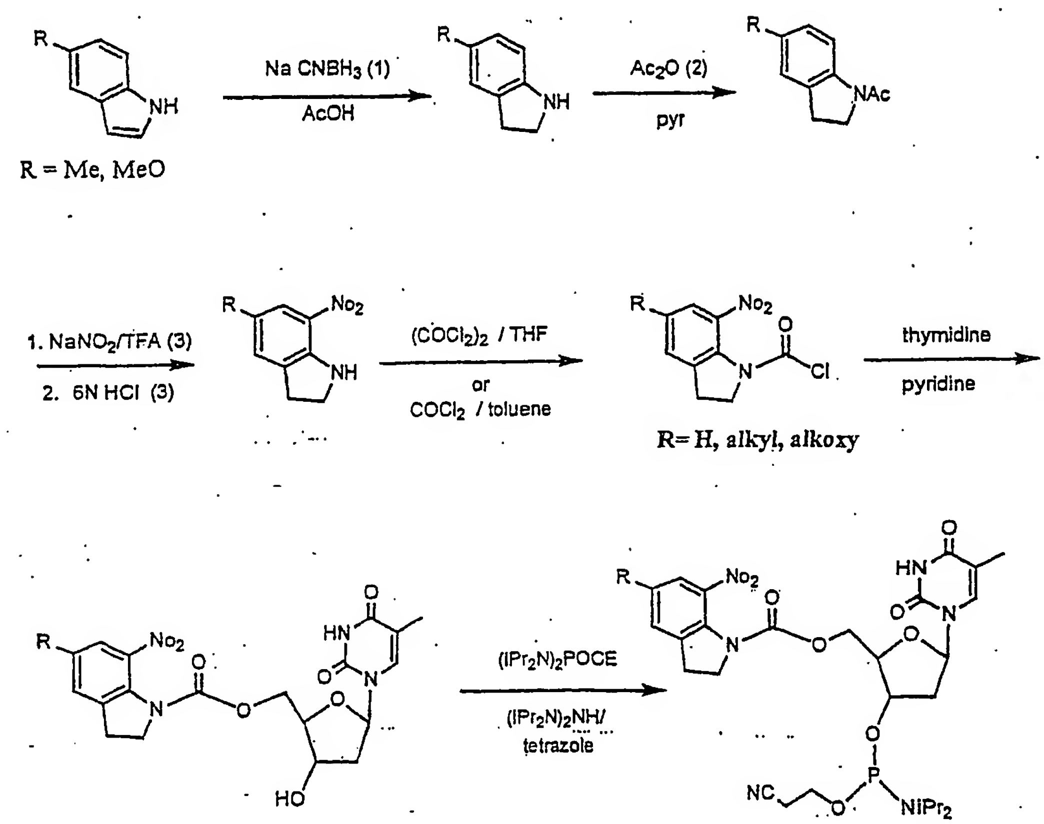
5'-TEMPOC-T-Phosphoramidite



1. Dyer, et al. JOC 64: 7988 (1999)
2. Tetrahedron Lett., 38(52), 8933-4 (1997)
3. McGall, et al. JACS 119: 5081 (1997)
4. triphosgene may work equally well for this step.
5. chloroformate can probably be used without purification.

Figure 3

Synthesis of NINOC-T-CEP



(1) Bromidge, et al. (1998) *J. Med. Chem.* **41:** 1598.
(2) (i) Brooker, LS, et al. (1953) *US Pat. 2,646,430*; (ii) Boekelheide, et at. (1954) *J. Org. Chem.* **19:** 504.; (iii) Bennet, et al (1941) *J.Chem. Soc.* **74:** 244.
(3) Mortensen, et al. (1996) *Org. Prep. Proc. Int.* **28:** 123.

Figure 4

Me2NPOC-T-CEP

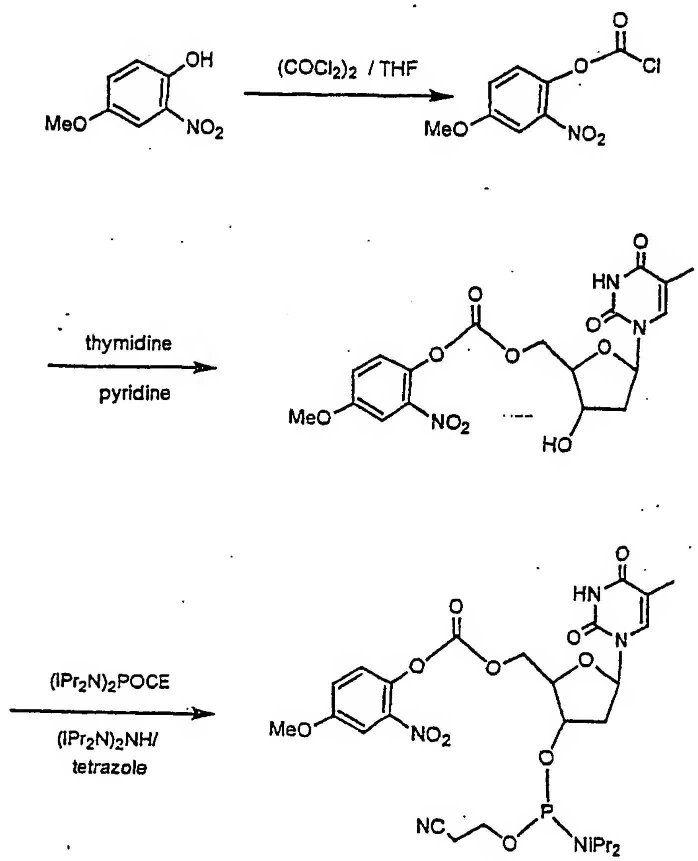
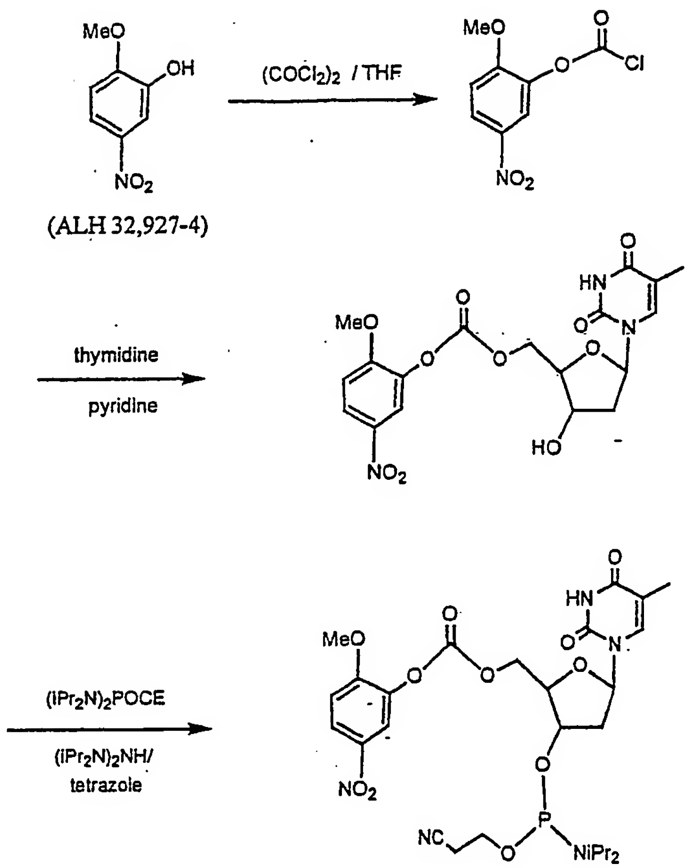


Figure 5

Me3NPOC-T-CEP



(ALH 32,927-4)

Figure 6

NP2POC-T-CEP

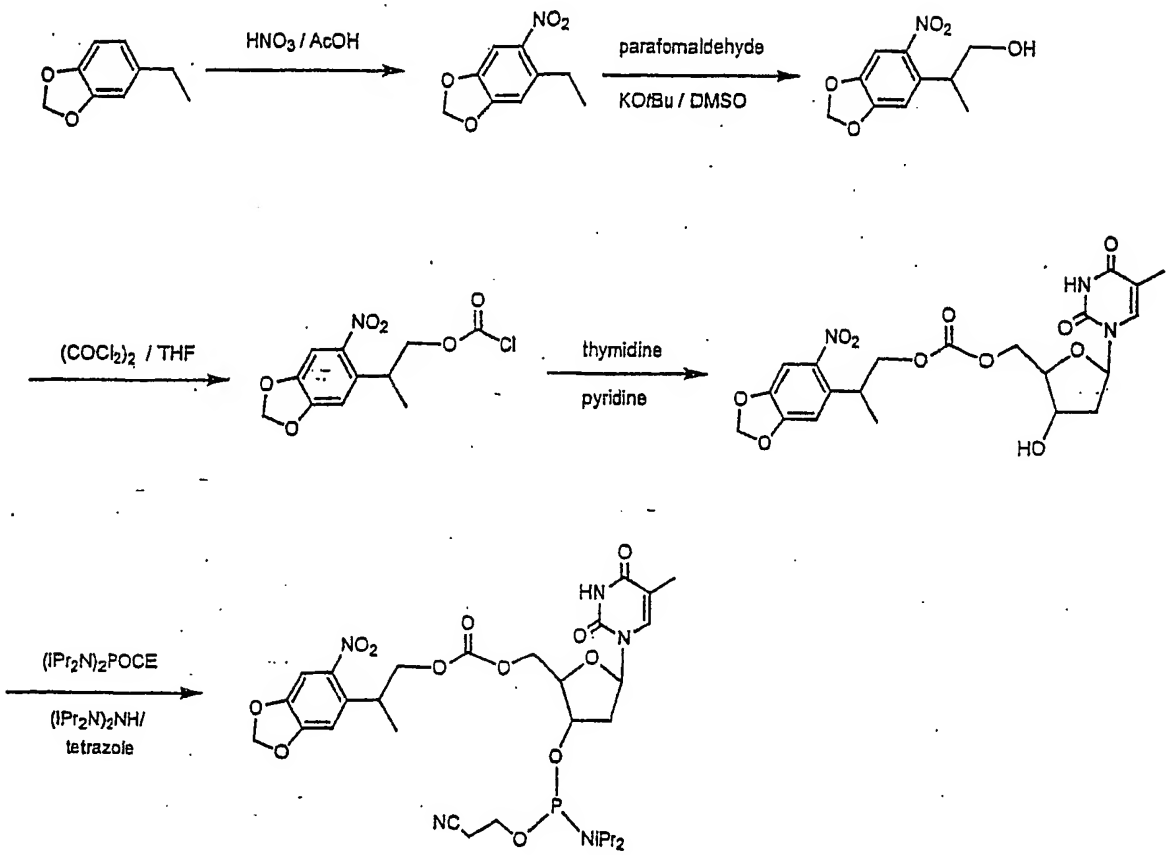


Figure 7

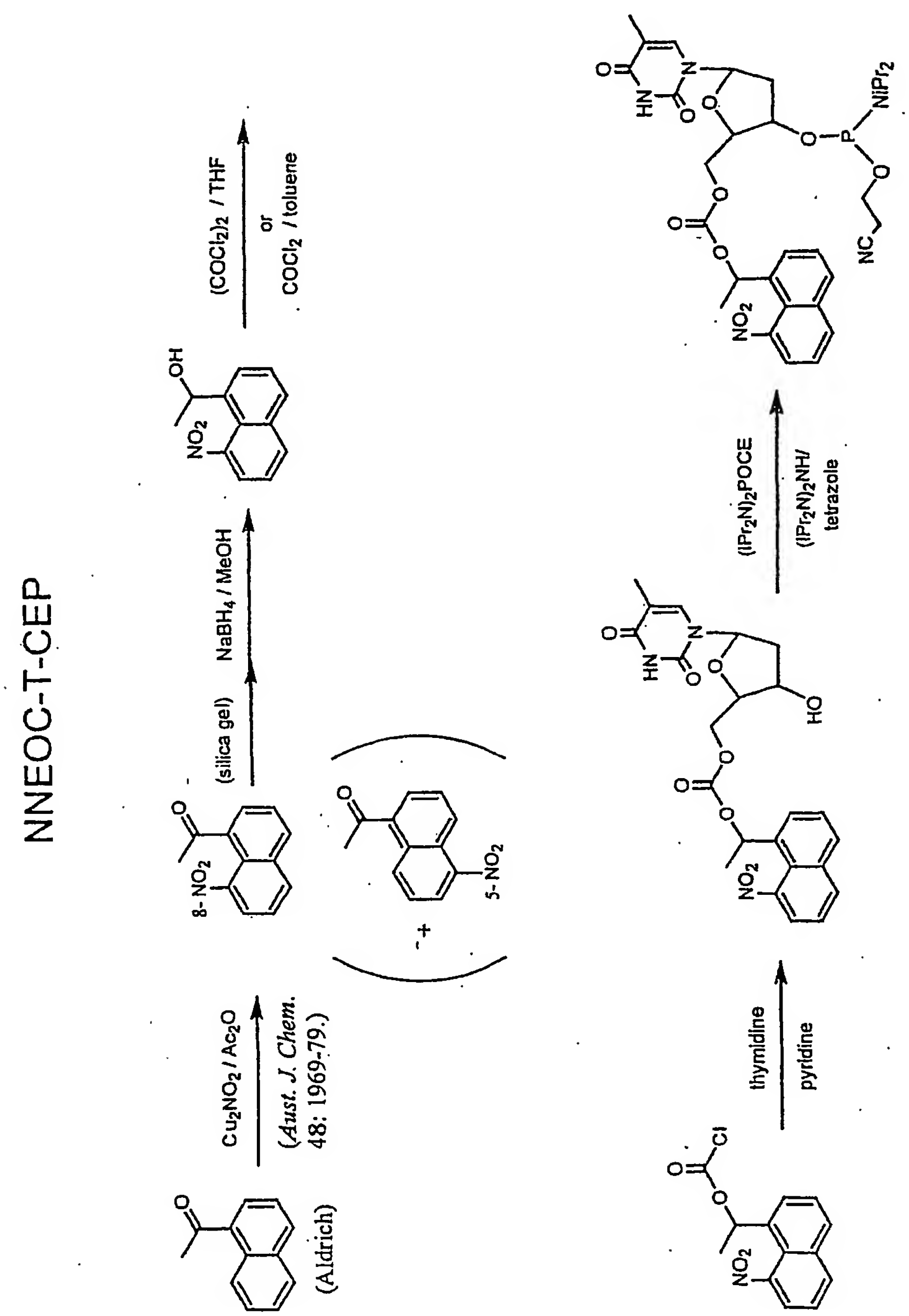
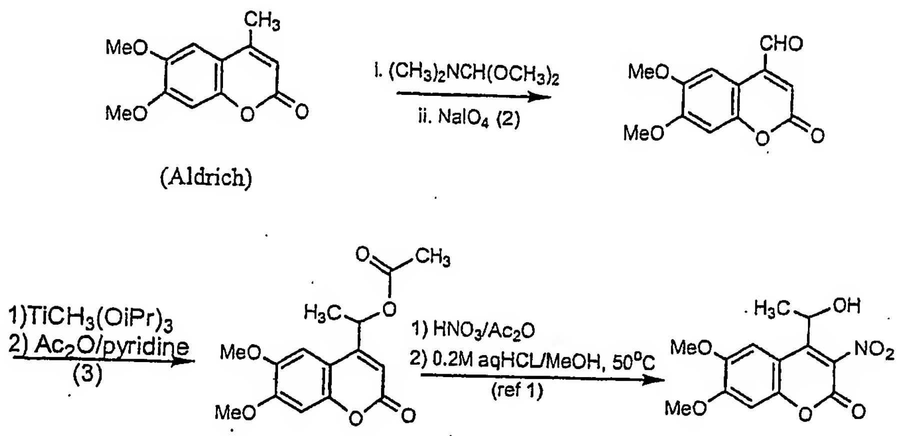

NNEOC-T-CEP
(Aldrich)
Cu_2NO_2 / Ac_2O
(*Aust. J. Chem.* 48: 1969-79.)
8- NO_2
5- NO_2
+
(silica gel)
$NaBH_4$ / MeOH
NO_2
OH
$(COCl_2)_2$ / THF
or
$COCl_2$ / toluene
Cl
thymidine
pyridine
HN
HO
$(iPr_2N)_2POCE$
$(iPr_2N)_2NH$/
tetrazole
NC
$NiPr_2$

Figure 8

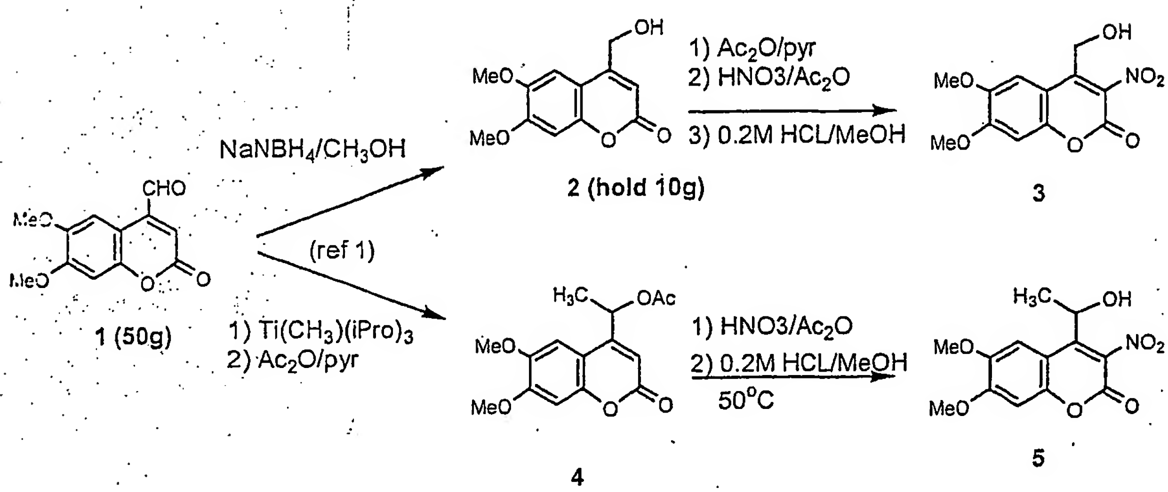
1-(3-nitrocoumarin-4-yl)ethyl alcohol

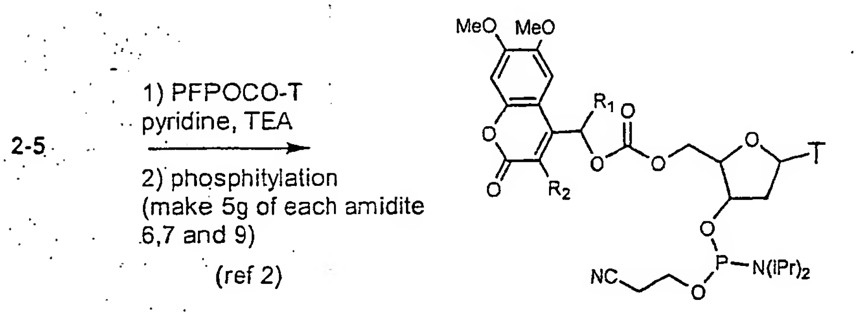


(1) Riesgo EC, et al (1996) *J. Org. Chem.* **61**: 3017.
(2) (1985) ***Chem. Ber.*** 118: 1421.
(3) Patel MG; et al. (1960) ***J. Indian Chem. Soc.*** 37(4): 227-30.

Figure 9

6,7- or 7,8- or 5,7-dimethoxycoumarin phosphoramidites



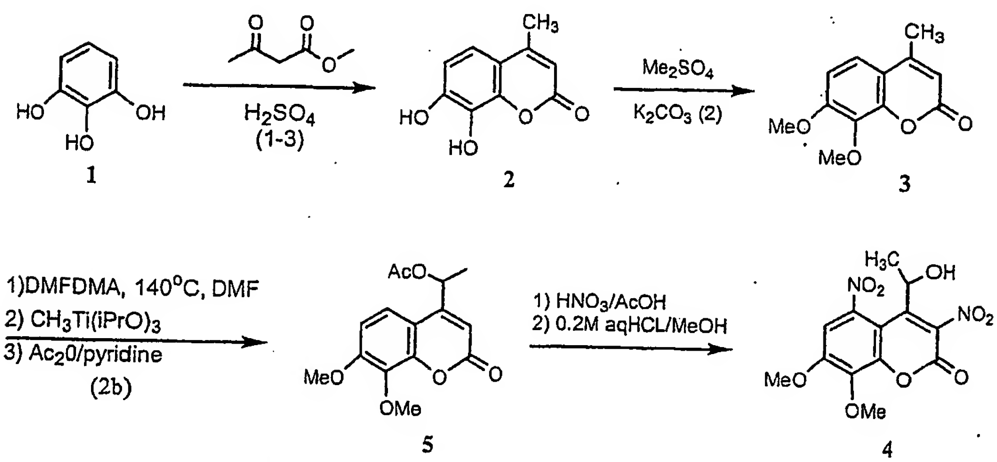


6 (R$_1$=H, R$_2$=H)
7 (R$_1$=H, R$_2$=NO$_2$)
8 (R$_1$=Me, R$_2$=H)
9 (R$_1$=Me, R$_2$=NO$_2$)

References and Notes
1. Affymetrix procedures
2. standard TriLink procedures; make 5g amidite of **6**,**7** and **9**.

Figure 10

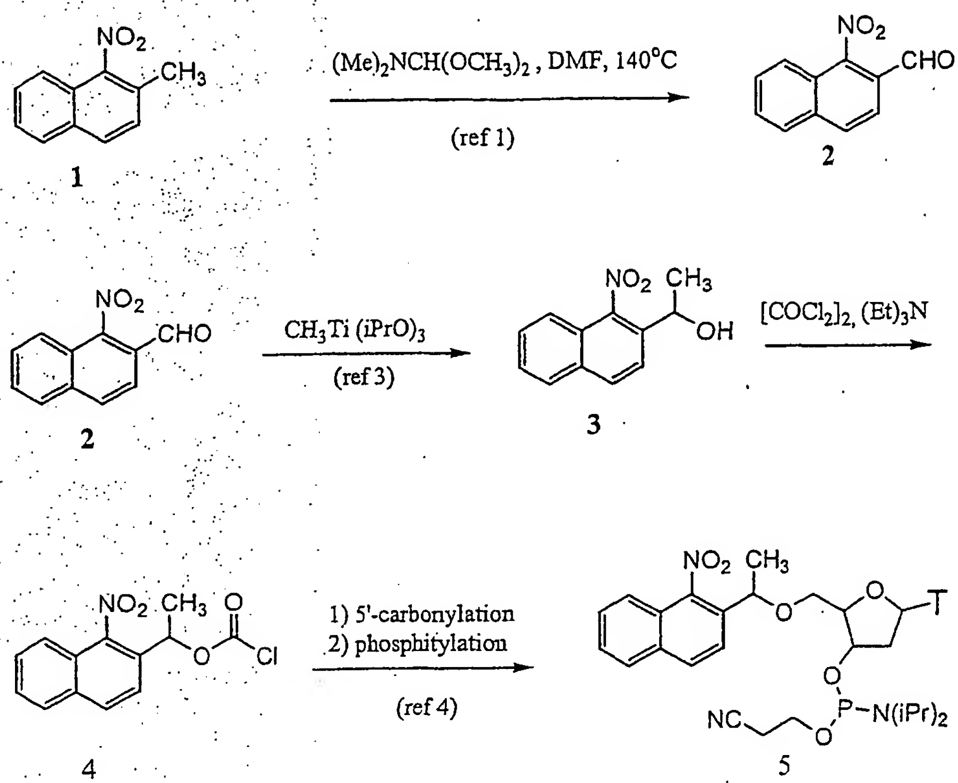
7,8-dimethoxy-5-nitrocoumarinyl-4-ethanol.2 (DMNCE)



References
(1) Ding, Q, et al. *Het. Commun.* **1997**, 3: 489.
(2) a) Ito K; Nakajima K, *J. Heterocycl. Chem.* **1988**, *25: 511-15* b) Baldwin,C., et al **1992**, *J. Org. Chem.* 57: 399-403
(3) Sethna S; Phadke R, *Org. React.* **1953**, *7: 1.*

Figure 11

(1,2)NNEOC-T-CEP

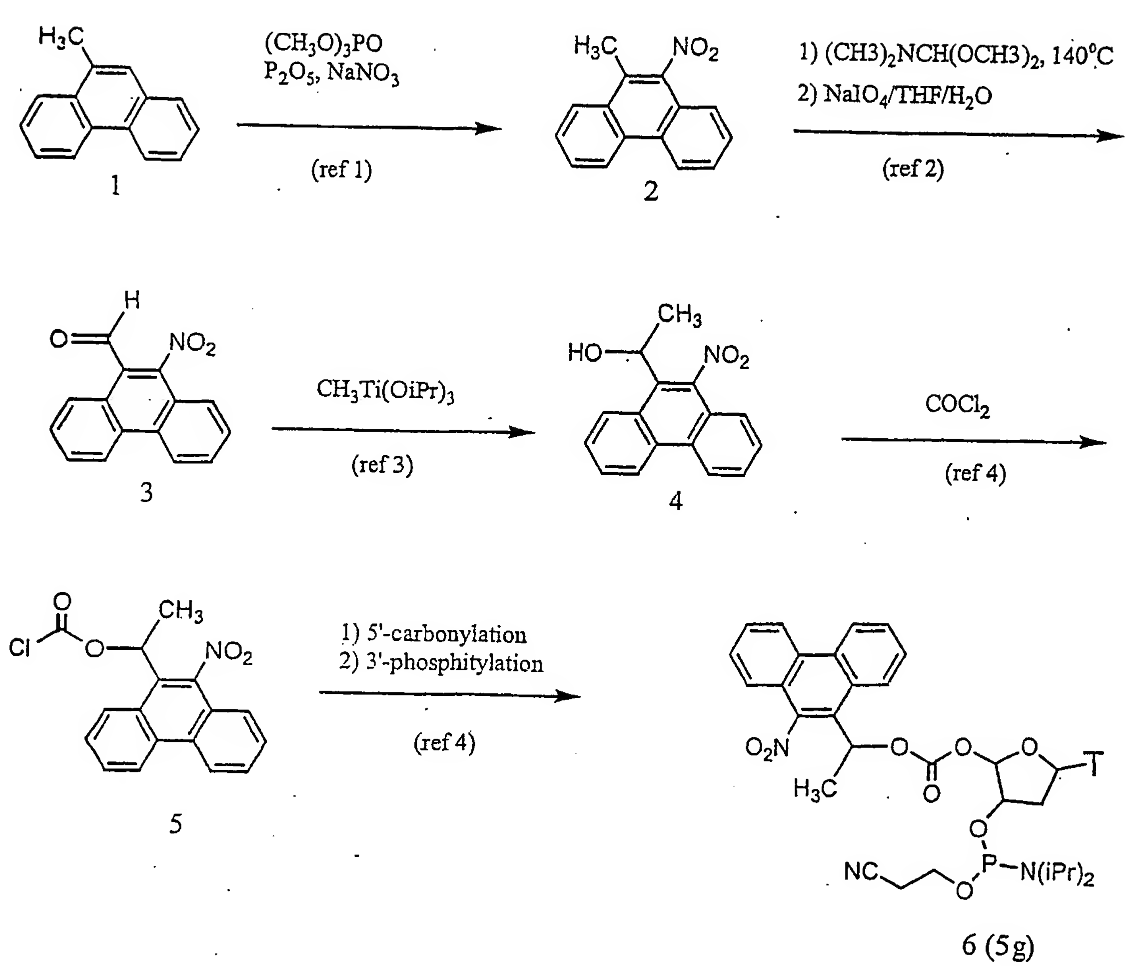


References

1) *JOC* **1996**, 61, 3017
2) *J. Fluorine Chem.* **1995**, 73, 21
3) *Chem. Ber.* **1985**, 118, 1421

Figure 12

(9,10)NPhenEOC-T-CEP



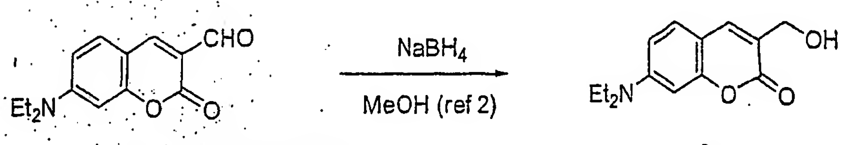
References

1) *Synthesis* 1976, 9, 621
2) *J. Org. Chem.* **1996**, 61, 3017
3) *Chem. Ber.* **1985**, 118, 1421
4) *J. Amer. Chem. Soc.* **1997**, 119, 5081

Figure 13

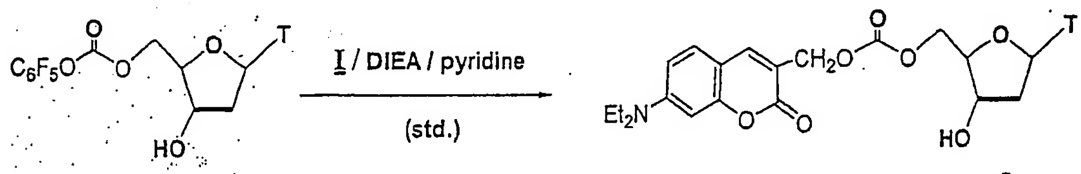
5'-(7-diethylaminocoumarin-3-yl)methyloxycarbonyl-T-CEP

(5'-DEACMOC-T-CEP)



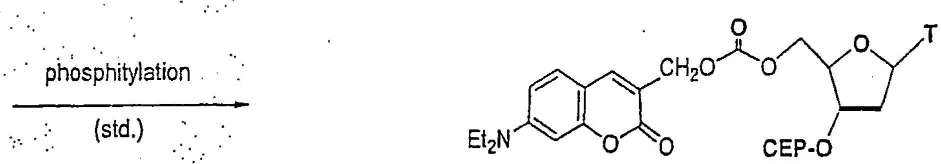
1(prepared as in ref 1)

2



4

5

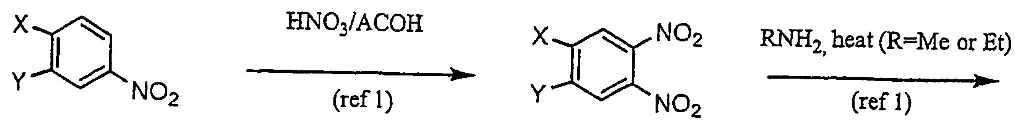


6

1. Machida, Minoru, et al (2000) *Chem. Pharm. Bull.48*, 1702.
2. Dutta, LN; et al., (1995) *Can. J. Chem.* 73(9): 1556-62.

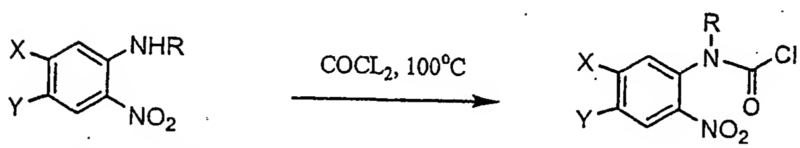
Figure 14

N-alkyl-4,5-substituted-2-nitroanalides



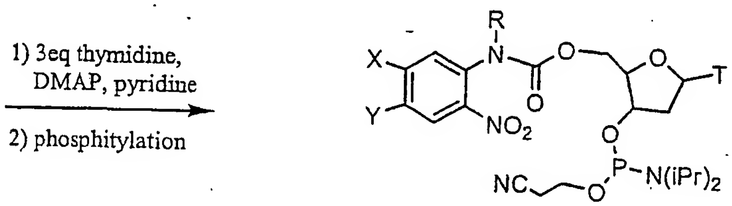
1 X=H, Y= OMe
2 X=Y= OMe
3 XY= methylenedioxy

4 X=H, Y= OMe
5 X=Y= OMe
6 XY= methylenedioxy



7 X=H, Y= OMe
8 X=Y= OMe
9 XY= methylenedioxy

10 X=H, Y= OMe
11 X=Y= OMe
12 XY= methylenedioxy

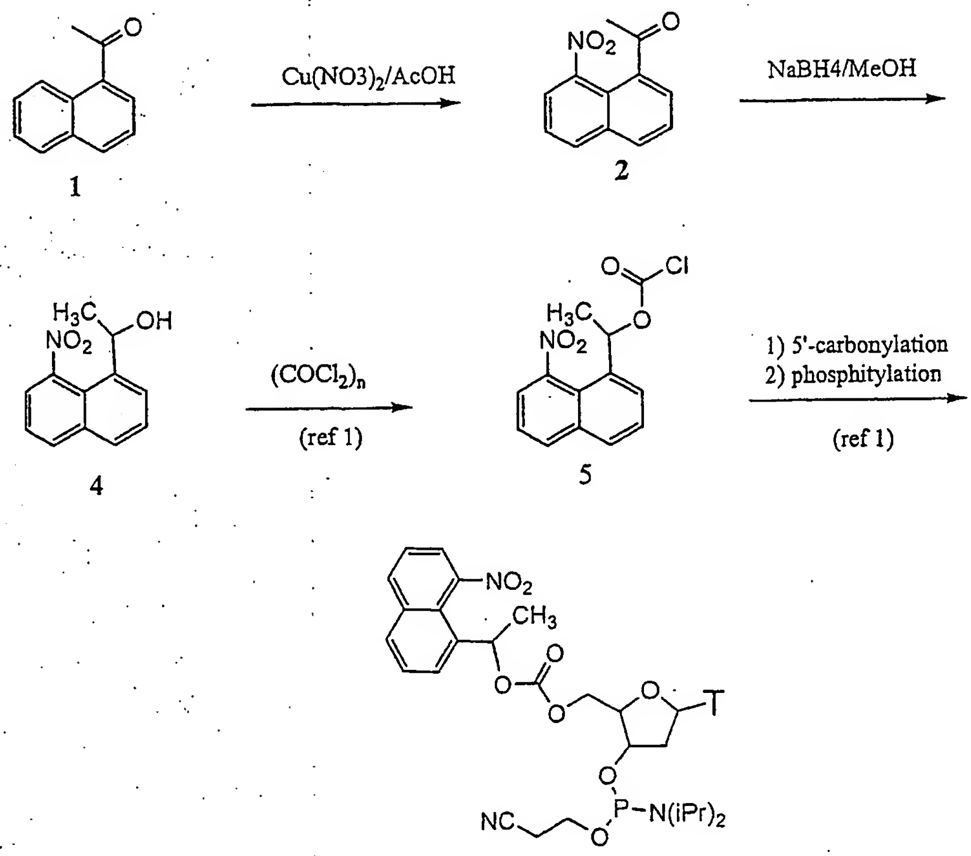


13 X=H, Y= OMe
14 X=Y= OMe
15 XY= methylenedioxy (specific compound made)

References
1) pat. appl. DE 86-3612665 (1986)]

Figure 15

(8,1)NNEOC-T-CEP



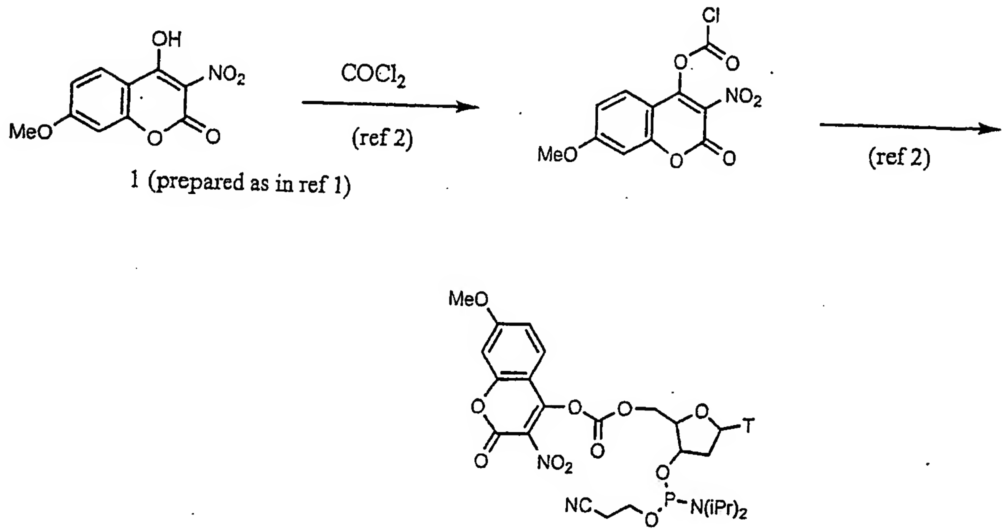
6

References

1) standard ChemGenes procedures

Figure 16

5'-(7-methoxy-3-nitrocoumarin-4-yloxycarbonyl)thymidine-3'-phosphoramidite



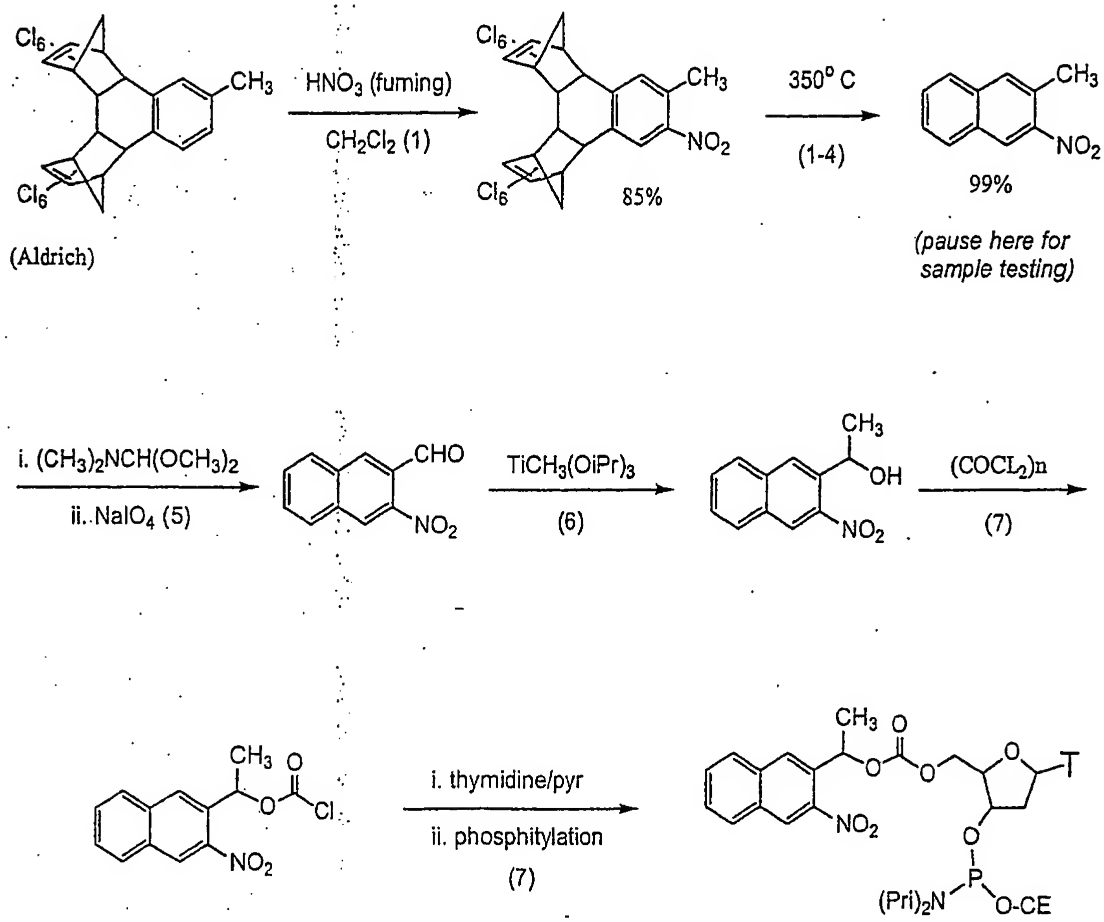
References

1) Buckel, D.R., et al., 1976, US 3,974,289

2) standard procedures found in McGall, G.H., et al, 1997, *J. Amer. Chem. Soc.*, 119, 5081.

Figure 17

(3,2)NNEOC-T-CEP

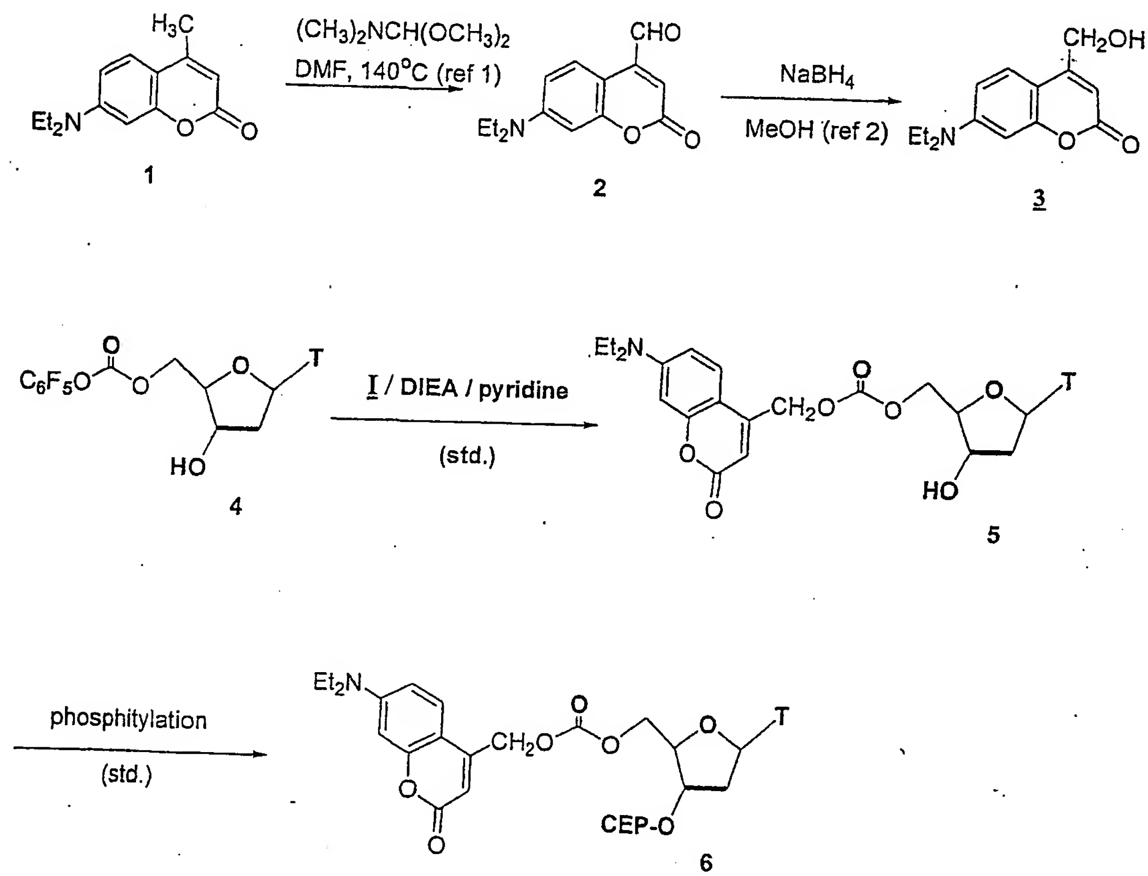


(1) Liu B (2000) ***J. Phys. Chem.*** **104: 1837.**
(2) Kelly TR, et al. (1994) *J. Amer. Chem. Soc.* **116**: 7072.
(3) Feynes, JG (1962) *J. Org. Chem.* **27**: 2614.
(4) Danish, AA, et al. (1954) *J. Amer. Chem. Soc.* **76**: 6144.
(5) Riesgo EC, et al (1996) *J. Org. Chem.* **61**: 3017.
(6) Reese M, et al. (1985) ***Chem. Ber.*** 118: 1421.
(7) Established procedures.

Figure 18

5'-(7-diethylaminocoumarin-4-yl)methyloxycarbonyl-T-CEP

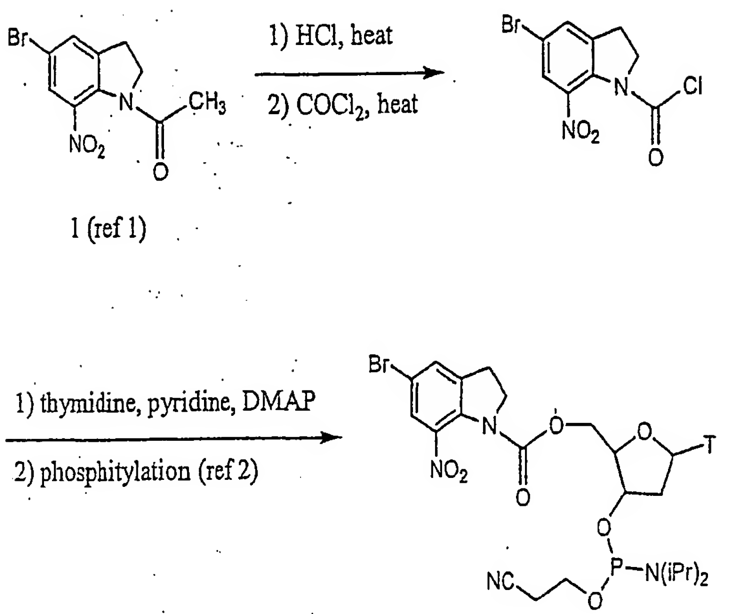
(5'-DEACMOC-T-CEP)



1. Thummel, R.P, et al (1996) *J. Org. Chem.*, 3017.
2. Dutta, LN; et al., (1995) *Can. J. Chem.* 73(9): 1556-62.

Figure 19

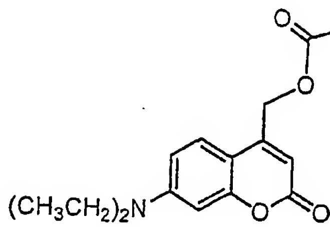
5-bromo-7-nitroindolinylcarbonyl-T-CEP
(BNIC)



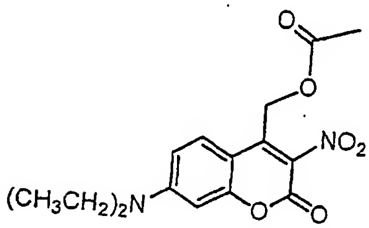
References

1) prepared according to procedures found in Christensen, J. B., et al. 1996, *OPPI Briefs*, 28, 123 and Gall, W. G. et al. 1955, *J. Org. Chem.*, 20, 1538.
2) McGall, G. H., et al. 1997, 119, 5081.

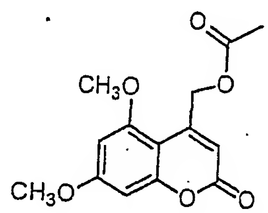
Figure 20



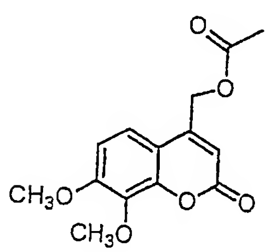
DEACMOC-74
7-diethylaminocoumarin-4-yl)methyloxycarbonyl



DEANCMOC-734
7-diethylamino-3-nitrocoumarin-4-yl)methyloxycarbonyl

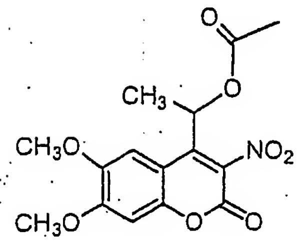


DEANCMOC-4
(5,7-dimethoxycoumarin-4-yl)methyloxycarbonyl

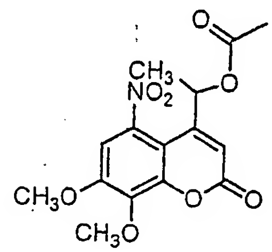


DEANCMOC-4
(7,8-dimethoxycoumarin-4-yl)methyloxycarbonyl

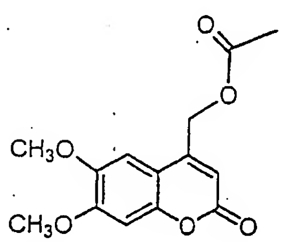
Figure 21A



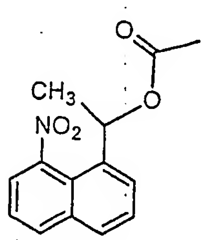
DMNCEOC-6734 :
1-(6,7-dimethoxy-3-nitrocoumarin-4-yl)ethyloxycarbonyl



DMNCEOC-7854
1-(7,8-dimethoxy-5-nitrocoumarin-4-yl)ethyloxycarbonyl

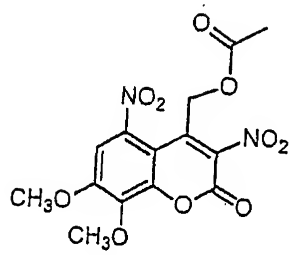


DMCMOC-674
6,7-dimethoxycoumarin-4-yl)methyloxycarbonyl

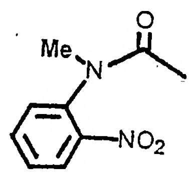


NNEOC-81 :
1-(8-nitronaphthalene-1-yl)ethyloxycarbonyl

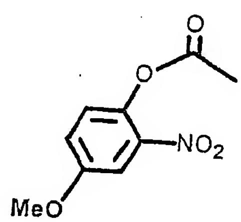
Figure 21B



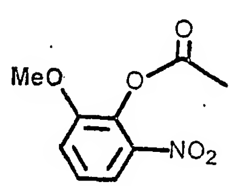
DMDNCEOC-78354
1-(7,8-dimethoxy-3,5-dinitrocoumarin-4-yl)ethyloxycarbonyl



MNAC

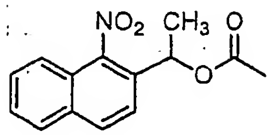


MNPOC-4

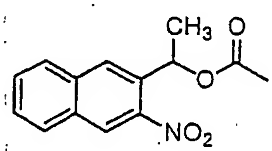


MNPOC-6

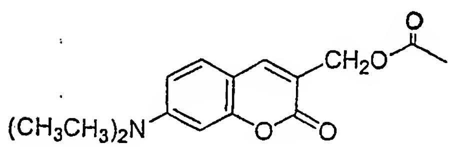
Figure 21C



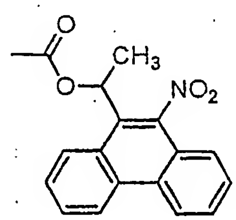
NNEOC-21 (
1-(2-nitronaphthalene-1-yl)ethyloxycarbonyl



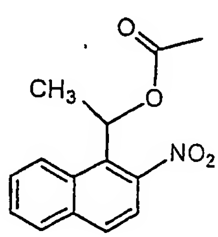
NNEOC-32
1-(3-nitronaphthalene-2-yl)ethyloxycarbonyl



DEACMOC-73
7-diethylaminocoumarin-3-yl)methyloxycarbonyl

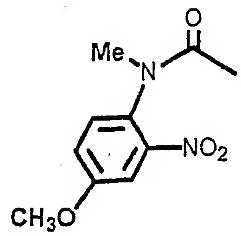


NPhEOC-109
(10-nitrophenanthren-9-yl)ethyloxycarbonyl

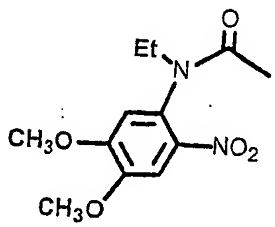


NNEOC-21
1-(2-nitronaphthalene-1-yl)ethyloxycarbonyl

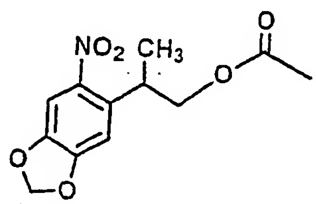
Figure 21D



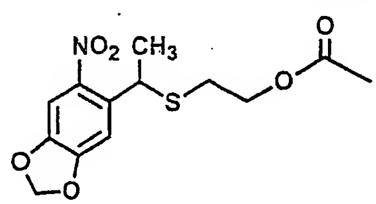
MMNAC-4
4-methoxy-2-nitro-N-methylanilinecarbonyl



DMNVAC-45
4,5-dimethoxy-2-nitro-N-ethylanilinecarbonyl



MNPPOC-45
2-(4,5-Methylenedioxy-2-nitrophenyl)propyloxycarbonyl



MeNPTEOC

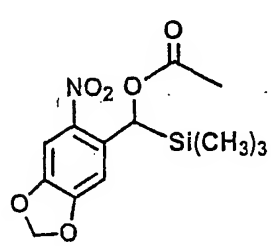
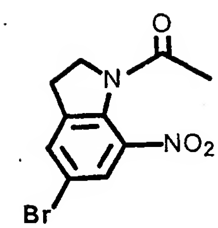
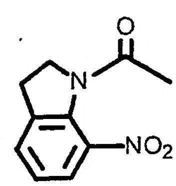


Figure 21E



BNIC
5-bromo-7-nitroindolinylcarbonyl



NIC
7-nitroindolinylcarbonyl

Figure 21F

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 March 2002 (14.03.2002)

PCT

(10) International Publication Number
WO 02/020150 A3

(51) International Patent Classification[7]: C07H 19/10, 21/00, C07K 17/06

(21) International Application Number: PCT/IB01/01650

(22) International Filing Date: 11 September 2001 (11.09.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/659,599 11 September 2000 (11.09.2000) US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US 09/659,599 (CIP)
Filed on 11 September 2000 (11.09.2000)

(71) Applicant (*for all designated States except US*): **AFFYMETRIX, INC.** [US/US]; 3380 Central Expressway, Santa Clara, CA 95051 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): **MCGALL, Glenn, H.** [CA/US]; 1121 Sladky Avenue, Mountain View, CA 94040 (US). **BARONE, Anthony, D.** [US/US]; 2118 Ellen Avenue, San Jose, CA 95125 (US).

(74) Agents: **TREANNIE, Lisa, M.** et al.; Hamilton, Brook, Smith & Reynolds, P.C., 530 Virginia Road, P.O. Box 9133, Concord MA 01742-9133 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report: 13 March 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/020150 A3

(54) Title: PHOTOCLEAVABLE PROTECTING GROUPS

(57) Abstract: Novel compounds are provided which are useful as linking groups in chemical synthesis, preferably in the solid phase synthesis of oligonucleotides and polypeptides. The compounds are generally photolabile and comprise protecting groups which can be removed by photolysis to unmask a reactive group. The protecting groups has the general formula (Y), wherein: (Y) is a chemical structure as shown the Figure. Also provided is a method of forming, from component molecules, a plurality of compounds on a support, each compound occupying a separate predefined region of the support, using the protected compounds described above.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 01/01650

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07H19/10 C07H21/00 C07K17/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07H C07K C07B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 10128 A (AFFYMAX TECH NV ;HOLMES CHRISTOPHER P (US); SOLAS DENNIS W (US); K) 11 May 1994 (1994-05-11) figures 1-6 ---	1,6,15
A	US 5 763 599 A (PFLEIDERER WOLFGANG ET AL) 9 June 1998 (1998-06-09) claim 1 ---	1,6,15

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 July 2002	29. 10. 2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Held, P

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GRIEGRICH H ET AL: "New photolabile protecting groups in nucleoside and nucleotide chemistry - synthesis, cleavage mechanisms and applications" NUCLEOSIDES & NUCLEOTIDES, MARCEL DEKKER, INC, US, vol. 17, no. 9-11, 1998, pages 1987-1996, XP002159918 ISSN: 0732-8311 the whole document -----	1,6,15

INTERNATIONAL SEARCH REPORT

national application No.
PCT/IB 01/01650

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-24 (partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-24 (partially)

   A compound represented by formula Y-X wherein Y is defined by the first Markush formula mentioned in claim 1; related methods

2. Claims: 1-24 (partially)

   A compound represented by formula Y-X wherein Y is defined by the second Markush formula mentioned in claim 1

3. Claims: 1-24 (partially)

   A compound represented by formula Y-X wherein Y is defined by the third Markush formula mentioned in claim 1; related methods

4. Claims: 1-24 (partially)

   A compound represented by formula Y-X wherein Y is defined by the fourth Markush formula mentioned in claim 1; related methods

5. Claims: 1-24 (partially)

   A compound represented by formula Y-X wherein Y is defined by the fifth Markush formula mentioned in claim 1; related methods

6. Claims: 1-24 (partially)

   A compound represented by formula Y-X wherein Y is defined by the sixth Markush formula mentioned in claim 1; related methods

7. Claims: 1-24 (partially)

   A compound represented by formula Y-X wherein Y is defined by the seventh Markush formula mentioned in claim 1; related methods

8. Claims: 1-24 (partially)

   A compound represented by formula Y-X wherein Y is defined by the eighth Markush formula mentioned in claim 1; related

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

methods

9. Claims: 31-45, 55-72

A compound represented by formula Y-X and related methods

10. Claims: 25-30, 46-54, 73-90 (all partially)

A compound represented by formula Y-X wherein Q2=O and Q4=O, and not covered by subjects 1,3,5 and 6; related methods

11. Claims: 25-30, 46-54, 73-90 (all partially)

A compound represented by formula Y-X wherein Q2=O and Q4=S or NR13, and not covered by subjects 1,3,5 and 6; related methods

12. Claims: 25-30, 46-54, 73-90 (all partially)

A compound represented by formula Y-X wherein Q2=S and Q4=O, and not covered by subjects 1,3,5 and 6; related methods

13. Claims: 25-30, 46-54, 73-90 (all partially)

A compound represented by formula Y-X wherein Q2=S and Q4=S or NR13, and not covered by subjects 1,3,5 and 6; related methods

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB 01/01650

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
WO 9410128	A	11-05-1994	AU	5449094	A	24-05-1994
			WO	9410128	A1	11-05-1994
US 5763599	A	09-06-1998	DE	4444996	A1	20-06-1996
			AT	215957	T	15-04-2002
			AU	692658	B2	11-06-1998
			AU	4386596	A	03-07-1996
			BR	9510498	A	30-11-1999
			CA	2207912	A1	20-06-1996
			CZ	9701836	A3	17-12-1997
			DE	59510162	D1	16-05-2002
			DK	797580	T3	17-06-2002
			WO	9618634	A2	20-06-1996
			EP	0797580	A2	01-10-1997
			FI	973643	A	09-09-1997
			HU	77176	A2	02-03-1998
			IL	116407	A	13-09-2001
			JP	11501287	T	02-02-1999
			NO	972754	A	11-08-1997

Form PCT/ISA/210 (patent family annex) (July 1992)